University of Alberta

Maintenance energy metabolism in non-pregnant and pregnant sows

by

Ryan Scott Samuel

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Animal Science

Department of Agricultural, Food and Nutritional Science

Edmonton, Alberta
Spring, 2008

Library and Archives Canada

Published Heritage Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et Archives Canada

Direction du Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 978-0-494-45880-8
Our file Notre référence
ISBN: 978-0-494-45880-8

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

ABSTRACT

Non-pregnant sows were fed approximately 1x the maintenance energy requirement (ME_m) or 2x ME_m. Half of the daily ration was offered in 16, ½-hourly meals followed by the remaining half in a single meal. Sows fed 1x ME_m had greater heat production (HP) than energy intake. Sows fed 2x ME_m had greater ($P<0.05$) HP than sows fed 1x ME_m and gained weight. The respiratory quotient (RQ) of sows fed 2x ME_m indicated lipogenesis. Calculated ME_m (506 kJ/$BW^{0.75}$) was greater than current recommendations. Sows had equally elevated HP on a 24 h basis when consuming either frequent small meals or a large meal.

The HP and RQ were measured on days 30, 45, and 105 of gestation in sows fed according to current recommendations and indicated excess energy was stored on day 45 by lipogenesis and energy intake was above requirement on days 30 and 45, but below requirement on day 105.

To my family: past, present, and future.

ACKNOWLEDGEMENTS

I would like to thank Dr. Ronald Ball and Dr. Paul Pencharz for their supervision during my MSc program and for peaking my interest in nutrition and metabolism. I appreciate the knowledge and guidance you have offered to me thus far as I continue my research career. I would also like to acknowledge Dr. Linda McCargar for serving as a member of my committee.

Special thanks for the scientific and technical assistance and knowledge provided by Dr. Soenke Moehn. Dr. Moehn designed and constructed, from individual components, the indirect calorimetry system that was used in this research. Dr. Moehn is the reason for the direction, organization, and success of this research.

I would like to acknowledge members of the Ball-Pencharz group and their contributions. Thank you to Drs. Kristine Urschel and Desmond Pink for introducing me to the lab and to Dr. Urschel for experimental and technical assistance, to Dr. George Komban for daily animal care and experimental and laboratory assistance, to Christine Pendlebury and Pamela Wizzard for experimental assistance, and to Dr. Jacob Attakora for laboratory assistance.

Thank you to the staff of the Swine Research and Technology Centre for your assistance and for making the SRTC a wonderful place to be and conduct research: Jay Willis, Janes Goller, Dianna Goller, and Tamara Staska. I would also like to thank Dr. Craig Wilkinson, Brenda Tchir, and Charlane Gorsak for their

assistance with animal care. Thank you to all of the staff and other graduate students of AFNS, especially to Jody Forslund.

Finally, I extend a huge thank you to my wife, Nadine, to my parents, and to the rest of my family for supporting my research aspirations.

TABLE OF CONTENTS

1.0 LITERATURE REVIEW

1.1	Energy metabolism	1
1.2	Reducing the energy barrier to metabolism	3
1.3	Enzyme pathways of metabolism	4
1.4	Basis for measuring heat production	6
1.5	Measuring heat production	7
1.6	Energy value of complex molecules	12
1.7	Metabolic rate – Definitions	14
1.8	Heat increment of feeding	15
1.9	Allometric scaling	17
1.10	Maintenance energy requirement – Definition	18
1.11	Maintenance energy requirement – Current values for swine	19
1.12	Maintenance energy requirement – Determination and calculation	23
1.13	Energy value of diets	25
1.14	Effect of feeding frequency on energy metabolism	27
1.15	Respiratory quotient	29
1.16	Adaptation to new energy intake	30
1.17	Improved productivity of modern, high producing sows	31
1.18	Summary	33
1.19	Literature Cited	34

2.0 RATIONALE, HYPOTHESES, AND OBJECTIVES

2.1 Rationale 39

2.2 Null hypotheses 41

2.3 Specific objectives 42

2.4 Literature Cited 43

3.0 THE 24 h HEAT PRODUCTION OF NON-PREGNANT SOWS FED 473 kJ/BW$^{0.75}$ AND 925 kJ/BW$^{0.75}$

3.1 Introduction 45

3.2 Materials and Methods 48
- 3.2.1 Animals and ethics approval 48
- 3.2.2 Diets, feeding, and housing 48
  - 3.2.2.1 Addition of Celite® (acid insoluble ash) 50
  - 3.2.2.2 Calculation of daily feed allowance 50
  - 3.2.2.3 Experimental feeding regimen 50
- 3.2.3 Body weight and daily gain 52
- 3.2.4 Indirect calorimetry 52
- 3.2.5 Chemical analyses 53
  - 3.2.5.1 Sample grinding 53
  - 3.2.5.2 Bomb calorimetry 53
  - 3.2.5.3 Acid insoluble ash content 54
  - 3.2.5.4 Crude fat content 55
  - 3.2.5.5 Neutral detergent fibre content 56
  - 3.2.5.6 Acid detergent fibre content 57
  - 3.2.5.7 Nitrogen and carbon contents 58
  - 3.2.5.8 Ash content 58
- 3.2.6 Calculations 58
  - 3.2.6.1 Digestibility 58
  - 3.2.6.2 Volumes of gases 59
  - 3.2.6.3 Heat production 59
  - 3.2.6.4 Respiratory quotient 60
  - 3.2.6.5 Maintenance energy requirement 60
- 3.2.7 Statistical analysis 61

3.3 Results 61
- 3.3.1 Sow body weight, daily gain, and feed intake 61
- 3.3.2 Nutrient digestibility 61
- 3.3.3 24 h heat production 62
- 3.3.4 Calculated maintenance energy requirement 62
- 3.3.5 Respiratory quotient 63

3.4 Discussion 64

3.5 Conclusions and implications 69

3.6 Literature Cited 70

4.0 THE HEAT INCREMENT OF FEEDING WITH RESPECT TO FEEDING FREQUENCY

4.1 Introduction 72

4.2 Materials and methods 75
4.2.1 Statistical analysis 75

4.3 Results 75
4.3.1 Feeding frequency and feeding level effect on heat production 75
4.3.2 Feeding frequency and feeding level effect on respiratory quotient 76

4.4 Discussion 76

4.5 Conclusions and implications 81

4.6 Literature cited 82

5.0 THE 24 h ENERGY EXPENDITURE OF PREGNANT SOWS AT EARLY-, MID-, AND LATE-GESTATION

5.1 Introduction 84

5.2 Materials and Methods 86
5.2.1 Feeding 86
5.2.2 Calculations 87
5.2.2.1 Digestibility 87
5.2.2.2 Heat production 87
5.2.2.3 Respiratory quotient 87
5.2.2.4 Energy intake/BW^x 87
5.2.2.5 HP/BW^x 88
5.2.2.6 Calculated litter weight 88
5.2.2.7 Calculated weight of products of conception 88
5.2.2.8 Calculated maternal BW 89
5.2.2.9 Maternal ME_m HP 89
5.2.2.10 Total BW ME_m HP 89
5.2.3 Statistical analysis 89

5.3 Results 90
5.3.1 Nutrient digestibility 90

5.4 Discussion 91

5.5 Conclusions and implications 98

5.6 Literature cited 99

6.0 GENERAL DISCUSSION AND CONCLUSION

6.1 Introduction 101

6.2 Changes of energy metabolism 101

6.3 Application of ME_m value 103

6.4 Conclusion 104

6.5 Literature Cited 105

APPENDIX

A.1 Introduction 106

A.2 Calorimetry system validation 106

A.3 Oxygen sensor details and the effect of temperature 107

LIST OF TABLES

Table 1.1 Literature estimates of ME_m for sows 21

Table 1.2 Comparison of sow productivity – 1998 & 2004 33

Table 3.1 Diet composition of dry sow ration 49

Table 3.2 Proximate analysis of the dry sow ration as-fed 49

Table 3.3 Effect of feeding level (473 vs. 925 $kJ/BW^{0.75}$) on nutrient digestibility by sows 62

Table 3.4 Pooled means for nutrient digestibility of non-pregnant sows 62

Table 3.5 Energy intake, heat production, and calculated ME_m for individual sows 63

Table 3.6 RQ of sows offered (473 $kJ/BW^{0.75}$ or 925 $kJ/BW^{0.75}$) during various prandial states (fasting, post-prandial, or meal eating) on the RQ of sows 64

Table 4.1 Mean 24 h heat production of sows by feeding level and prandial state 76

Table 4.2 Respiratory quotient of sows by feeding level and feeding frequency 76

Table 5.1 Proximate analysis of the diet as-fed during gestation 86

Table 5.2 Nutrient digestibility least square means and P-values for gravid sows on three 'days' of gestation 90

Table 5.3 Pooled means of nutrient digestibility for gravid sows on three 'days' of gestation 90

Table 5.4 Means for daily gain (g/d) of gravid sows between study 'days' of gestation and body weight, 24 h HP, daily energy intake, RQ, metabolic HP, and metabolic energy intakes of gravid sows on study 'days' of gestation 91

Table 5.5 Calculated daily gain of the litter and weight on respiration days of the litter, products of conception, and maternal body 91

LIST OF FIGURES

Figure 1.1 Energy partition of food energy 25

Figure 1.2 Phenotypic trends in Canadian Yorkshire and Landrace populations 32

Figure 3.1 Graphical representation of the data collection 'period' by experimental feeding regimen 51

Figure 3.2 Heat production of sows fed 473 kJ/$BW^{0.75}$ (1x ME_m) versus 925 kJ/$BW^{0.75}$ (2x ME_m) according to collection 'period' and feeding regimen 'state' 51

Figure A.1 Schematic of the indirect calorimetry system 108

LIST OF ABBREVIATIONS

Acetyl-CoA – acetyl co-enzyme A
ADF – acid detergent fibre
AIA – acid insoluble ash
ATP – adenosine triphosphate
BMR – basal metabolic rate
BW – body weight
cal – calorie
CF – crude fat
CO_2 – carbon dioxide
CP – crude protein
CTAB – cetyl trimethylammonium bromide
CV – coefficient of variation
d – day
DC – digestibility coefficient
ddH_2O – double-distilled water
DE – digestible energy
dH_2O – distilled water
DigLys – digestible lysine
EE – ether extract
ER – energy retention
$FADH_2$ – reduced form of flavin adenine dinucleotide
FHP – fasting heat production
FMR – field metabolic rate
G-6-P – glucose-6-phosphate
GE – gross energy
GTP – guanosine triphosphate
h – hour
H_2SO_4 – sulfuric acid
HIF – heat increment of feeding
HP – heat production
J – joules
k – kilo
k_m – efficiency of energy utilization for maintenance
k_{pf} – efficiency of energy utilization for protein and fat deposition
L – litres
Lys – lysine
M - mega
ME – metabolizable energy
MEm – metabolizable energy requirement for maintenance
mol - mole
NADH + H+ – reduced form of nicotinamide adenine dinucleotide
NADPH – reduced form of nicotinamide adenine dinucleotide phosphate
NDF – neutral detergent fibre

NE – net energy
NE_m – net energy requirement for maintenance
NO_x – nitrous oxide compounds
NPRQ – non-protein respiratory quotient
RMR – resting metabolic rate
RQ – respiratory quotient
RT – room temperature
SBM – soy bean meal
SMR – standard metabolic rate
TCA – tricarboxylic acid cycle
TEF – thermic effect of feeding
USP – United States Pharmacopeia
V_{CH4} – volume of methane
V_{CO2} – volume of carbon dioxide
V_{O2} – volume of oxygen

1.0 LITERATURE REVIEW

1.1 Energy metabolism

Energy is required by all living organisms for life processes, including respiration, circulation, maintenance of core body temperature and concentration gradients, and to perform work including the transport and turnover of small and large molecules of body tissue. Metabolism serves to provide the energy necessary for life processes and work by the break down of complex organic molecules and eventual oxidation of the resulting products (IOM, 2005). In accordance with the First Law of Thermodynamics, the energy derived from metabolism is neither created nor destroyed, but instead transferred or changed in form. Potential energy is transferred when chemical bonds are broken and new bonds are formed (Campbell, 1996). In fact, the overall goal of energy metabolism is to release stored energy from organized forms of matter such as carbohydrates, fats, and, to a lesser extent, proteins (Rolfe and Brown, 1997). These exergonic reactions proceed by releasing energy from the bonds of complex molecules. The bonds of the waste products, carbon dioxide and water, store less energy than the complex molecules from food. Therefore, the energy released from the breakdown of food must be trapped in a useable form; otherwise it would be lost as heat into the environment (IOM, 2005).

Adenosine triphosphate (ATP) is a compact storage molecule for the energy released due to the break down of complex molecules during metabolism. A number of intermediates, in addition to ATP, are used to distribute energy including: "GTP, NADH, NADPH, mitochondrial proton gradient, and the plasma membrane [Na^+]

gradient" (Rolfe and Brown, 1997). The break of a single phosphate-phosphate bond of ATP releases 31 kJ/mol of ATP into the environment surrounding the reaction. However, within the cellular environment, a total of 54 kJ/mol of ATP can be released by the break of a single phosphate-phosphate bond (Campbell, 1996).

As previously discussed, energy metabolism breaks down complex molecules into less complex intermediates and energy is released. The chemical reactions of energy metabolism are reversible in the presence of their products and the resulting equilibrium would halt further energy transfers. However, the Le Chatelier principle states that chemical reactions can be forced into disequilibrium by the addition or removal of product or reactants (Laidler and Meiser, 1999). In the case of metabolism, the desired direction for the disequilibrium is toward the product and continued energy transfers. Removal of product from the vicinity of the reaction forces the reaction forward. Often products of a reaction become reactants in a subsequent reaction. As a result, disequilibrium is maintained, thus allowing energy transfer reactions to continue (IOM, 2005).

Metabolism mirrors the progression of nature towards a state of increasing entropy, but in a controlled manner. Through step-wise breakdown of complex molecules from food into the waste products carbon dioxide and water, energy is transferred to intermediates for transport throughout the body. Therefore, in order for an organism to survive, it is necessary to consistently take in energy producing molecules and to capture the products of energy transfer reactions, thereby supporting the metabolic processes of the body and allowing an organism to live (IOM, 2005).

1.2 Reducing the energy barrier to metabolism

There is a different energy barrier associated with the metabolism of different substrates. The complexity of the molecule and strength of the bonds within the molecule will affect the energy required to break down that molecule. More complex molecules, such as proteins, require greater energy input to facilitate breakdown than do less complex molecules, such as carbohydrates (IOM, 2005).

Enzymes are essential for metabolism for two key reasons: 1) enzymes lower energy barriers, and 2) enzymes allow metabolism to proceed in a step-wise manner. The overall reactions of metabolism are spontaneous because of the increase in disorder (Second Law of Thermodynamics) and because they are exergonic (negative Gibbs' energy[1]). However, in order for the reactions to proceed, there is an energy cost associated with bond breaking and reforming. The energy required to initiate a reaction is termed the 'activation energy'. Enzymes are able to lower the energy required to initiate a reaction by physically acting on the reactants, i.e. substrates. Enzymes are also able to regulate metabolism by releasing energy through step-wise chains and cycles. This is essential for the efficient transfer of energy from the complex molecules undergoing metabolism to the energy transfer intermediates and, ultimately, the whole body (IOM, 2005).

[1] Negative Gibbs' energy values indicate energy released into the environment surrounding the reaction. Positive Gibbs' energy values indicate that energy must be absorbed from the environment for the reaction to occur (Laidler and Meiser, 1999).

1.3 Enzyme pathways of metabolism

The key to the efficient release of energy from complex molecules is that energy is not released in a single step. Instead, a number of steps are needed as part of three chains and one cycle. These, in order for glucose metabolism, are: glycolysis, pyruvate oxidation, tricarboxylic acid cycle (TCA), and the respiratory chain (Campbell, 1996).

Glycolysis is the anaerobic, step-wise breakdown of glucose occurring in the cytosol of cells. Other sugars may also be metabolized by glycolysis, provided they can be converted into intermediates of glycolysis by appropriate enzymes. The initial steps of glycolysis are priming reactions. A single phosphate group of an ATP molecule is attached to glucose almost immediately upon entering the cell. This has three effects: 1) a concentration gradient is maintained thus forcing more glucose to enter the cell, 2) now negatively charged, glucose-6-phosphate (G-6-P), cannot passively diffuse across the plasma membrane, and 3) G-6-P is more unstable than glucose. Because phosphorylated glucose (G-6-P) is different than glucose and cannot diffuse through the plasma membrane due to its negative charge, concentration equilibrium between the inside and outside of the cell cannot be established. Therefore, more glucose travels into the cell by passive diffusion down the concentration gradient to where the glucose is low. Phosphorylation serves to 'activate' molecules, in this case glucose, by making the molecule more unstable and therefore lowering the activation energy required to break bonds. The amount of Gibbs' energy released is not, however, affected. A second phosphate group from a second ATP is added after isomerization of G-6-P to fructcose-6-phosphate, further decreasing the stability of the molecule. The ATP balance of glycylosis is as follows: 4 ATP/mol of glucose produced – 2 ATP/mol of glucose used

in the priming reactions. Therefore, 2 molecules of ATP/mol of glucose are produced by glycolysis as well as 2 molecules of pyruvate (Garrett and Grisham, 1999).

Pyruvate, the end product of glycolysis, still contains a significant amount of energy. Pyruvate is not used directly in the TCA cycle. Instead, pyruvate is a significant source of the Acetyl-Coenzyme A (acetyl-CoA) used in the TCA cycle. Acetyl-CoA is a two carbon metabolite formed from pyruvate by oxidation of the carboxyl carbon and release as CO_2. Energy transferred from the carbon-carbon bond breaking is trapped by NADH + H^+. Oxidative decarboxylation of pyruvate to acetyl-CoA serves to bridge glycolysis and the TCA cycle in the metabolism of glucose (Garrett and Grisham, 1999).

The TCA cycle involves a number of enzyme catalyzed steps to oxidize the carbon backbone of carbohydrates, fats, and proteins. Different substrates can enter the TCA cycle at different points, depending on the availability of enzymes to produce cycle intermediates from the substrates (Salway, 1999). Acetyl-CoA is the two carbon metabolite that enters the TCA cycle by joining to the four carbon oxaloacetate, yielding a six carbon citrate molecule. In the first energy producing step of the TCA cycle, the carboxyl carbon from acetyl-CoA is cleaved off and released as CO_2. Again, the energy stored in this bond is transferred and trapped in NADH + H^+. In the next step, the second carbon from acetyl-CoA is cleaved and released as CO_2. If the released CO_2 remained in the mitochondria, these energy transfer reactions would be reversible leading to equilibrium, according to Le Chatelier's principle. However, the CO_2 quickly diffuses away (dissolved in plasma or red blood cells) or is altered in form (carbamino complexes in plasma or red blood cells or bicarbonate in red blood cells) to maintain the

disequilibrium of life. The TCA cycle continues generating energy storage intermediates that can be used in the respiratory chain to generate ATP (Garrett and Grisham, 1999).

The energy transfer intermediates GTP, NADH + H^+, and $FADH_2$ are produced by the TCA cycle, but, as discussed earlier, ATP is the main energy transfer molecule within cells (Lipmann, 1941). Oxidative phosphorylation, the final step in glucose metabolism, is the group of energy transfer reactions that serve to produce ATP in the respiratory chain. An organized series of proteins and coenzymes make up the chain of redox reactions. Because the proteins and coenzymes are able to exist in two oxidation states, both the electrons and the protons are removed from the energy storage intermediates. As the electrons are transferred through the chain, the oxidation states of the proteins and coenzymes quickly change back and forth. Electrons are transferred because each protein or coenzyme in the chain has slightly greater affinity for electrons than the previous step in the chain. The protons are used to establish a concentration gradient across the mitochondrial membrane. The proton gradient is used to drive ATP synthase for the production of ATP. Ultimately, the electrons and protons are transferred to O_2 thus forming the product of combustion, H_2O (Garrett and Grisham, 1999)

1.4 Basis for measuring heat production

Metabolism serves to efficiently release the energy stored in complex molecules through enzyme catalyzed, step-wise chains and cycles. However, some of the energy is inevitably lost during the numerous energy transfer reactions. Energy cannot be created

or destroyed, but only transferred to a different form. Therefore, energy that is not captured in ATP is released as heat. Heat represents the most disorganized form of energy, and is therefore favoured, according to the Second Law of Thermodynamics. All forms of energy can normally be converted entirely into heat without any energy loss. Therefore, the energy stored in complex molecules can be converted into heat by either uncontrolled combustion or enzyme-catalyzed oxidation. Measurement of the heat of chemical reactions is known as calorimetry. Changing the energy into heat through combustion in a bomb calorimeter, for example, is the standard method to determine the energy content of the substance (Kleiber, 1987). The heat production (HP) due to the enzyme catalyzed reactions of metabolism can be measured by either direct or indirect calorimetry.

Measurement of the heat released due to the oxidation of complex molecules by an animal dates back to 1778 when Adam Crawford designed a combustion calorimeter. This was the first example of a direct calorimeter; the amount of heat released due to the animal's metabolism was measured directly by the increase in temperature of a given amount of water surrounding the calorimeter. In 1899, Atwater and Rosa "demonstrated that heat production and work of human beings is derived from the chemical energy of [sic] katabolized material" (Kleiber, 1987).

1.5 Measuring heat production

Energy input less energy output equates to the energy balance of a system, process, or subject. In the case of living organisms, sustained negative energy balance can lead to death because the disequilibrium of life cannot be maintained. The energy

balance of living organisms can be determined using calorimetry. Energy input can be measured using bomb calorimetry to quantitate the energy of food. Energy output or expenditure can be measured as HP, either directly or indirectly, along with bomb calorimetry of waste products.

Studies of energy metabolism require a measurement of energy balance. Specifically, the energy retention (ER) must be measured in some way. The HP, and, by subtraction from energy intake, the ER, may be measured by two common techniques: direct calorimetry, indirect calorimetry, or the ER may be measured directly by the comparative slaughter or carbon and nitrogen balance method.

The latter method requires strict measurements of total carbon and nitrogen intake and output, known more commonly as a balance experiment. Nutrient balance experiments can be achieved by housing and feeding the animals individually in metabolism crates. The amount of food eaten must be known very precisely, which can be difficult in animal experiments as variable amounts of food may be wasted during eating. Metabolism crates allow complete collection of urine and feces. However, complete collection of waste products is never attained in reality and thus overestimates carbon and nitrogen retention, leading to 3 – 4% lower estimate of HP compared to indirect calorimetry (Christensen, 1988).

In comparative slaughter experiments, the energy contents of a sub-sample of representative whole carcasses are determined. This is accomplished by sub-sampling from a homogenous mixture of the ground whole carcass and determining the energy content by complete oxidation in a bomb calorimeter (Close and Stanier, 1984). Comparative slaughter determines the ER in the body tissues during the experimental

period. The difference between the final and the initial energy content of the animal carcasses is taken to represent ER over that period. The initial body composition and energy content of the animals is determined by a sample of animals at the beginning of the experimental period (Noblet and Le Dividich, 1982) or by data from previous research (McNutt and Ewan, 1984). Therefore, the comparative slaughter method has the advantage of directly associating carcass composition with energy metabolism (McNutt and Ewan, 1984). However, disadvantages of the comparative slaughter technique include the competing factors of cost (i.e. labour) and precision. The relative balance between the two must be determined based upon the expected treatment effect, statistical confidence level, and applicability of predicting measurements to determine the number of animals required for the whole of the experiment and representative sub-samples (Kempster et al, 1982). Kempster et al (1982) stated that "live animal assessment in experiments and population studies involving carcase [sic] evaluation is often overlooked" and could "increase the precision of the study considerably". Thus, information collected from live animals, through techniques of *in vivo* body composition measurements (e.g. ultrasonic) and calorimetry, are preferable to comparative slaughter.

Direct calorimetry measures the change in ambient temperature of the surrounding air as a result of heat produced by metabolism (Kleiber, 1987). There are two main classes of direct calorimeters: adiabatic or isothermal and conduction. The former class allows for removal and measurement of the heat generated by the subject within the chamber. Therefore, the sensible heat loss of the animal is measured directly. In the latter class, heat is allowed to flow from the chamber through walls of known

thickness and thermal conductivity. The temperature difference between the inside and outside layers of the chamber is proportional to the heat flow across the chamber wall. Naturally, the setup of a direct calorimeter is time consuming and complicated by the need for precision construction and the choice of appropriate materials. For example, the number of junctions required for the measurement of temperature difference is "in the order of 9,000 for the animal container" of a conduction direct calorimeter (Blaxter, 1971).

The major concept underlying indirect calorimetry dates back to 1780 when it was elucidated by Lavoisier and Laplace, from their studies of animal metabolism, that animal heat production is primarily a result of oxygen consumption. This concept is not surprising given the role of oxygen in the metabolism of complex molecules as the final electron and proton acceptor of the respiratory chain (Kleiber, 1987). Therefore, indirect calorimetry uses the biological concept of respiration and associated measurable gas exchange to calculate HP. The subject does not need to be complexly housed and in large animal and human studies, often only the head is within a hood. Gas exchange can be measured continuously (e.g. ventilated hood[2] or respiration chamber[3]) or intermittently (i.e. Douglas Bag method[4]), which is often favoured in human studies. Brouwer (1965) further refined the calculation of HP (kJ) from gas exchange with his formula:

$$16.175 \times V_{O2} + 5.16 \times V_{CO2} - 2.42 \times V_{CH4} - 5.90 \times \text{Urinary Nitrogen content (g)}$$

[2] An airtight plastic hood is placed over the subject's head and ventilated by a small adjustable fan creating a slight negative pressure that draws air into the hood and through to the analyzers continuously during the experiment (Simonson and DeFronzo, 1990).

[3] An airtight chamber is constructed large enough to contain the entire animal. A slight negative pressure is maintained within the chamber to draw fresh air into the chamber and through the analyzers continuously during the experiment (Simonson and DeFronzo, 1990).

[4] Expired air is collected in a Douglas Bag or similar air-tight container using a three-way respiratory valve during the experiment and then later analyzed for $[O_2]$ and $[CO_2]$ (Simonson and DeFronzo, 1990).

The ER can be measured directly from the balance method, changes in *in vivo* body composition, or comparative slaughter. Alternatively, HP and, therefore, ER, can be measured by the two calorimetry methods discussed. The carbon and nitrogen balance method does not require intricate instrumentation for measurements of heat (direct calorimetry), but carbon gas evolution (CO_2 and CH_4) must be quantified and the balance measurements must be made with high precision (Christensen et al, 1988). Both balance experiments and comparative slaughter become increasing difficult with the size of the animal with respect to housing (metabolism crate) as well as complete collection of waste products for the former and obtaining representative sub-samples for analysis in the latter case. Also, the calculations involve a number of assumptions of whole body and tissue compositions. Direct calorimetry is the simplest in concept because the heat loss is measured directly as sensible heat, but the construction and maintenance of the chambers and associated calculations are complicated. Indirect calorimetry requires accurate measurements of gas exchange, with particular attention to O_2, but the collection does not necessarily require a respiration chamber. Instead, subjects can be trained to exhale air through a mask or mouthpiece. However, these techniques are not practical for measurements lasting more than 30 minutes in duration (Simonson and DeFronzo, 1990). The construction of an indirect calorimetry chamber is not complicated by requirements for specific materials, as in direct calorimetry. An important advantage of continuous measurements of gas exchange is that changes of energy metabolism can be detected on minute to minute basis. Therefore, hood or chamber indirect calorimetry methods are the preferred techniques for measurements of ER.

1.6 Energy value of complex molecules

The energy available from the metabolism of complex molecules is different depending on the substrate being metabolized. There are differences in the amount of stored energy and the energy required to metabolize substrates, therefore resulting in different useful energy values from the different metabolic substrates. The three major classes of dietary substrates are carbohydrates, lipids, and protein (IOM, 2005).

The carbohydrate, starch, is the major energy fraction of diets fed to swine (NRC, 1998). Starch is a polymer of glucose and the major energy storage molecule in plants. The energy inputs required for the polymerization of glucose and concomitant heat of hydrolysis are low due to the simplicity of the linkages. Because the polymerization and hydrolysis are enzyme-catalyzed, the required energy inputs are further reduced. The energy available from starch is not much greater than the energy available from glucose monomers. Starch has a heat of combustion ranging from 17.2 kJ – 17.3 kJ/g (Kienzle et al. 2001) and the heat of combustion for glucose is 15.9 kJ/g (Garrett and Grisham, 1999). Starch is composed of two different types of polysaccharide α-linkages: α(1→4) and α(1→6). Amylose contains only α(1→4) linkages and is, therefore, linear. Amylopectin contains both α(1→4) and α(1→6) linkages, resulting in a highly branched structure. Starch typically consists of 10 – 30% amylase and 70 – 90% amylopectin. Animals store a certain amount of glucose as glycogen, which is branched in an analogous manner as amyopectin, mostly in the liver. The advantage of the highly branched structure of glycogen is the ability of enzymes to quickly attach (glycogenesis) and cleave (glycogenolysis) glucose monomers from the multiple branches (Garrett and Grisham, 1999).

While polysaccharides are useful for major energy storage in plants, because of the comparatively low energy density, they are not the preferred storage molecule for animals. Instead, lipids are the major energy storage molecule in animals (Avram et al. 2005a,b). The energy density of body lipid is greater than twice that of starch with a heat of combustion of 39.6 kJ/g (ARC, 1981). This energy reservoir of triacylglycerols can be accessed during times of reduced energy intake through enzyme-catalyzed hydrolysis known as β-oxidation. The process of β-oxidation serves to cleave off two carbon units as acetyl-CoA that can be further oxidized in the TCA cycle for energy as well as transfer energy to $FADH_2$ and NADH. The resulting triacylglycerol, shortened by two carbons, is available for further β-oxidation (Garrett and Grisham, 1999).

Under normal circumstances, the vast majority of the energy requirement of an animal is met by oxidation of dietary carbohydrates and lipids. The remaining energy demand is met by the oxidation of proteins, specifically the carbon skeletons of amino acids. Proteins are composed of long chains of individual amino acid units bonded in linear arrangements. The major purpose of amino acids is to provide the necessary building blocks for protein synthesis. Protein synthesis is increased during growth, but occurs continuously in the body due to the process of protein turnover (IOM, 2005). Therefore, metabolism of proteins is centered on efficiently releasing individual amino acids for incorporation into body protein. Because mammals cannot store individual amino acids, except as a part of protein, amino acids in excess of requirements are oxidized for energy (IOM, 2005). The unique nature of each of the 20 amino acids requires a unique metabolic pathway for oxidation, but the pathways converge at 7 metabolic intermediates (Garrett and Grisham, 1999). The heat of combustion for body

protein is 23.7 kJ/g, with individual amino acids contributing different energy values to the total (ARC, 1981).

1.7 Metabolic Rate – Definitions

To allow comparisons within and between experiments, the HP of an individual must be measured under defined metabolic conditions. Three major metabolic conditions will be discussed here: standard metabolic rate (SMR), field metabolic rate (FMR), and resting metabolic rate (RMR).

The SMR is defined as "the steady-state rate of heat production by a whole organism" provided that "the individual is an adult and is awake but resting, stress free, not digesting food (prior food intake being at or around maintenance level), and maintained at a temperature that elicits no thermoregulatory effect on HP". A considerable amount of energy is expended by an individual under conditions of SMR. The measured HP under the conditions of SMR has commonly been accepted to represent the fixed maintenance or basal energy requirement of that individual, to which additional energy requirements for other metabolic processes may be added. However, in practice, the summation of energy requirements does not hold true due to changes in the metabolic rates of individual tissues in response to different energy demands (Rolfe and Brown, 1997).

The FMR is greater than the SMR owing to the fact that the strict standardized conditions are not adhered to. The conditions of FMR are meant to better represent the actual metabolism and requirements of an individual on a daily basis. Therefore, feeding and muscle use are not restricted thus causing an increase in metabolic rate

above the SMR. Because the extent that the FMR exceeds the SMR depends on conditions, it is difficult to predict one from the other (Rolfe and Brown, 1997).

The RMR is measured during the time an individual is resting (i.e. no muscle use) but still actively digesting a meal. Therefore, the RMR is part of the FMR (Rolfe and Brown, 1997). Because sows are primarily fed once per day and, except for consuming the meal, do not participate in significant physical activity, the RMR would represent a significant portion of the total daily HP.

1.8 Heat increment of feeding

Enzymes represent the means to the efficiency and operation of metabolism. Naturally there is an energy cost to producing these enzymes. The heat increment of feeding (HIF) is a measure of the rise in HP, above the SMR, associated with digesting a consumed meal. The HIF is due, in part, to the energy required to generate the necessary enzymes for metabolism of the meal and its substrates (Baldwin and Smith, 1974).

The generation of heat represents an inefficiency of metabolism because energy is lost to the environment. The HIF is defined as the increase in HP above the resting level following consumption of a standard meal. The goal of metabolism is to release energy from substrates for use by the living organism for further metabolic processes. However, as previously discussed, there is an energy barrier to the metabolism of substrates. The activation energy can be reduced by enzymes, but the enzymes themselves require energy to be produced. In fact, Baldwin and Smith (1974) assigned 18% of the increase in HP following a meal to the production of enzymes. Further, they

assigned 30% of the increase in HP to the production of fat from glucose, 14% to the production of glycogen from glucose, 24% to active transport of molecules across cell membranes, and 5% due to hydrolysis of carbohydrates in the gut. Rolfe and Brown (1997) suggested that synthesis of storage compounds, ingested food being used to generate ATP, and hormonal stimulation of the sympathetic nervous system are all partially responsible for the increase in HP. Other researchers (Milligan and Summers, 1986; Kelly and McBride, 1990) suggested that the majority of the HIF is due to increases in Na^+/K^+-ATPase activity and protein turnover. The Na^+/K^+-ATPase enzyme (EC 3.6.3.9) is common to virtually all cells and functions to maintain the concentration and electrical gradient of normal cells. This is of particular importance for the resting and action potentials of muscle and nerve cells. The concentration gradient is important as the driving force behind a number of active transport proteins (IOM, 2005). Milligan (1971) noted between 10 – 17 % "of the O_2 uptake of noncontracting muscle was expended in Na^+/K^+ transport".

Soucy and Leblanc (1999) explain that there are two phases to the HIF. The first phase, termed the cephalic phase, is due to sensory stimulation by the sight, smell, and taste of food. This sensory phase is mediated by the sympathetic nervous system through hormonal stimulation and direct nervous connection via the vagus nerve (Rolfe and Brown, 1997). The second phase, the gastrointestinal phase, gradually replaces the cephalic phase after approximately 40 minutes.

The HIF can be divided into short- and long-term effects. The short-term effect of the HIF is an increase of up to 25% of the RMR peaking 1 – 2 hours post-prandial in humans (Rolfe and Brown, 1997). Ramonet et al. (2000) reported the short-term effect

of HIF in pigs peaked 2 – 3 hours post-prandial and fell to 50% between 6.35 and 7.47 hours after meal feeding of a low and high fibre diet, respectively. LeBlanc and Diamond (1986) reported that the mere presence of food resulted in equivalent metabolic HIF responses in dogs. In their experiment, animals were only allowed to see and smell a meal, but were not allowed to actually consume the meal. The animals mounted a HIF response equivalent to their previous meal size without the benefit of caloric intake. They, therefore, proposed that increasing feeding frequency without adaptation would be energetically inefficient as a result of the significant HIF response to the meal, regardless of size or actual consumption.

1.9 Allometric scaling

Biological features follow universal rules of scaling that cover the 21 orders of magnitude of size from single cell organisms to whales (West et al. 2000). Metabolic rates and ME_m are often reported relative to the metabolic liveweight of swine, where metabolic liveweight is the BW raised to the power of ¾ (ARC, 1981). The metabolic liveweight exponent allows comparison across different species of adult energy and nutrient requirements (White and Seymour, 2005). The desire for a standard relationship based on measurement of BW was centered on the fact that other measurements are more difficult than BW. Brody (1945) cited work by Sarrus and Ramaeux from 1838 in which it was suggested that metabolic rate was proportional to $BW^{2/3}$, based on a relationship to body surface area. Determination of body surface area is complicated, however, by the measurements of either linear size or volume. The relationship, $BW^{2/3}$, was further supported by the work of Max Rubner in 1883 and was

accepted for almost 50 years (White and Seymour, 2005). Max Kleiber reported in 1932 the discovery that the relationship between BW and metabolic rate was a value equal to 0.75 ± 0.01 (West et al. 2000). The power ¾ was adopted for a number of reasons, not the least of which was the ease of slide rule calculations (White and Seymour, 2005). More recent investigations of growing pigs (Tess et al. 1984a; Tess et al. 1984b; Thorbek et al. 1984; Noblet et al. 1989) calculated, by linear regression, exponents from experimental data rather than accepting the ¾ exponent (Holmes and Breirem, 1974). Specifically, Noblet et al. (1989) reported that ME_m was on average constant per $kg^{0.60}$, regardless of sex, genotype, or weight class differences between 20 to 100 kg BW for pigs. Exponents ranged from 0.55 to 0.65, where the lower values were for barrows or heavy pigs, and those calculated for faster growing animals (i.e. younger and males) were higher. Therefore, it appears more appropriate to use the power of 0.60 for growing swine. For adult swine, no investigations questioning the validity of the ¾ exponent, or proposing a new exponent, were available at the time of writing. Experiments are therefore required to determine the correct BW exponent for adult swine.

1.10 Maintenance energy requirement – Definition

The ME_m is defined as the energy required by the body for the obligatory metabolic processes to maintain body function and body temperature with moderate physical activity. Obligatory metabolism includes digestion, assimilation, and transport of nutrients, and the production and release of wastes (Wenk et al, 1980). Therefore, the

intake of metabolizable energy (ME) leading to zero energy retention (ER) is the maintenance energy requirement (ME_m) (ARC, 1981).

1.11 Maintenance energy requirement – Current values for swine

Current estimates of the energy requirements for swine were summarized by the NRC (1998). The reported mean for the daily maintenance energy requirement (ME_m) of 106 kcal ME/kg $BW^{0.75}$ was based on ten literature values ranging from 92 to 160 kcal ME/kg $BW^{0.75}$. Of the ten experimental values used to define the daily energy requirement for all swine, four experiments used early-weaned piglets (Böhme et al, 1980; Noblet and Le Dividich, 1982; Campbell and Dunkin, 1983; Close and Stanier, 1984), two experiments were on normally (i.e. more than 21 days suckling) weaned piglets (McNutt and Ewan, 1984; Gädeken et al, 1985), and a further three values were reports based on growing pigs 20 to 120 kg BW (Whittemore, 1976; Wenk et al, 1980; Noblet et al, 1985). The additional reference is the review of ME_m by ARC (1981). From a number of sources over a large body weight range of 2 to 180 kg live weight, ARC (1981) estimated the daily ME_m for swine to be 458 kJ/kg $BW^{0.75}$. The suggested ME_m for sows, based on the reviews of ARC (1981) and NRC (1998) was 458 kJ/$BW^{0.75}$ and 106 kcal/kg $BW^{0.75}$ (equivalent to 444 kJ/kg $BW^{0.75}$), respectively.

Only 9 reports were found in the literature that specifically measured the ME_m for sows (Table 1.1). Reports by Lodge et al (1979) and Noblet and Close (1980) were investigations comparing the partition of ME intake at several stages of pregnancy to non-pregnant animals. Body weight gain was greater for pregnant animals, in both studies, due to the growth of products of conception. Noblet and Close (1980) indicated

that, by separation of total gain into maternal and reproductive portions, weight gain of the pregnant animals was not different in early (i.e. 47 and 49 days) and mid (75 and 68 days) gestation compared to non-pregnant animals at equal energy and protein intakes. They did report, however, that weight gain of pregnant animals in late (98 days) gestation was lower than occurred in non-pregnant animals of similar age. Further, Noblet and Close (1980) reported little difference in the efficiency of energy utilization for maintenance (k_m) between the pregnant and non-pregnant animals. The ME_m and corresponding k_m values were calculated by linear regression, based on measured HP by calorimetry. Their ME_m values were 432, 420, and 411 $kJ/BW^{0.75}$ from the three different equations and no differences between pregnant or non-pregnant animals were noted. Lodge et al. (1979) used comparative slaughter to partition ME intake into maternal and reproductive portions. Similarly, Lodge et al. (1979) reported no effect of pregnancy on ME_m, "other than can be accounted for by the increase in body weight". Lodge et al. (1979) also reported no differences in efficiency of energy utilization for maintenance for pregnant animals compared to non-pregnant animals.

There are seven other literature estimates of sow ME_m shown in Table 1.1, however reports on primiparous animals and of animals not housed in thermoneutral environments should be interpreted with caution. Primiparous animals have not achieved adult body weight or body composition. Therefore, ME_m values measured from these animals do not necessarily reflect the requirements of older, more developed, multiparous animals. Primiparous animals have a significant growth potential; the

Table 1.1 Literature estimates of ME_m for sows

ME_m (kJ/$BW^{0.75}$)	Weight (kg)	Parity	S[1]	Comments	Reference
418	133 – 219	Primiparous	Pr	average from early (30-50 d), middle (50-80 d), and late (95-110 d) gestation; calorimetry	Noblet and Etienne, 1987
422	105 – 203	Primiparous	Pr	Calorimetry	Close et al, 1985
420	105 – 203	Primiparous	NP	Calorimetry	Close et al, 1985
427	100 – 162	Primiparous	Pr	Calorimetry	Burlacu et al, 1983
513	115 – ?	Primiparous	Pr	applied energy coefficients of Thorbek (1975); comparative slaughter	De Wilde, 1980
502	115 – ?	Primiparous	NP	applied energy coefficients of Thorbek (1975); comparative slaughter	De Wilde, 1980
407	116 – 200	Primiparous	Pr	average from early (40-60 d), middle (60-80 d), and late (90-110 d) gestation; calorimetry	Noblet and Close, 1980
409	114 – 154	Primiparous	NP	average from early (40-60 d), middle (60-80 d), and late (90-110 d) gestation; calorimetry	Noblet and Close, 1980
452	130 – 180	Primiparous	Pr	average from d 56 and d 112 gestation; comparative slaughter; summer	Lodge et al., 1979
452	130 – 161	Primiparous	NP	average from d 56 and d 112 gestation; comparative slaughter; summer	Lodge et al., 1979
661	118 – 146	Primiparous	Pr	average from d 56 and d 112 gestation; comparative slaughter; winter	Lodge et al., 1979
640	120 – 132	Primiparous	NP	average from d 56 and d 112 gestation; comparative slaughter; winter	Lodge et al., 1979
753		Primiparous	NP	housed at 5 °C; comparative slaughter	Hovell et al, 1977
476		Primiparous	NP	housed at 5 °C; comparative slaughter	Hovell et al, 1977
530[2]		Primiparous	Pr	comparative slaughter	Hovell et al, 1977
444	128 – 202	Primiparous	Pr	housed at 18 °C; calorimetry	Holmes and McLean, 1974
385	128 – 202	Primiparous	NP	housed at 23 °C; calorimetry	Holmes and McLean, 1974
418	168 – 227	Multiparous	Pr	Assumed ME_m of 100 kcal	Verstegen et al, 1971

[1]: S = Status; Pr = pregnant; NP = non-pregnant; [2]: $BW^{0.85}$.

effects of which on ME_m estimates cannot be easily determined. Likewise, estimates derived from animals housed above or below temperatures of a thermoneutral environment do not necessarily reflect the requirements of other animals. Because these reports do not estimate the effect of temperature on the estimated ME_m, there is no way to correct the estimates for the effect of environmental temperature.

A review of swine heat production by Brown-Brandl et al (2004) revealed that daily HP "data for greater than 90 kg pigs" was insufficient. Equally important, Brown-Brandl et al (2004) calculated that HP had increased 18.1% during the period 1984 to 2002, based on the equations from Tess et al. (1984a,b). They cited the change in average body composition of the animals over that period as the reason for the increase in HP. Specifically, there was an increase in lean tissue mass and a reduction of whole body fat. Wenk et al (1980) also suggested that there has been an increased ME_m in modern swine due to an increased rate of lean tissue turnover. Tess et al. (1984a,b) associated a 2.1% increase in lean tissue percentage with increased HP of 18.7%. Thus, modern breeds of pigs require greater energy supply simply to support the maintenance of body tissues and obligatory metabolic processes.

Increased HP by swine has not been accounted for in current agricultural building designs (Brown-Brandl et al. 2004). The American Society of Agricultural Engineers standards (2003) refer to experiments performed by Bond et al. (1959) for the parameters required to calculate adequate ventilation for modern pig production. Brown-Brandl et al (2004) calculated an increase in daily HP of swine of approximately 1% per year over an 18 year period, based on the increase in lean tissue percentage over the same period. Therefore, in order to optimize the growing conditions for swine with

respect to both energy requirements and adequate ventilation and building design, actual measurement of the HP of modern swine is required. Specifically, due to their central importance to pork production and the lack of data, the HP of modern, high producing sows should be measured.

1.12 Maintenance energy requirement – Determination and calculation

Calculating ME_m from experimental results requires knowledge of the ER of the experimental animals. The ER can be determined either directly, by comparative slaughter or carbon-nitrogen balance, or indirectly by measuring HP. The methods to calculate ER have been discussed previously (see 1.5 Measuring heat production). No technique, however, gives direct information about the HP associated with protein or fat accretion as components of growth.

The two common methods to determine the daily ME_m of pigs are comparative slaughter and indirect calorimetry. Both have been used to determine the energy retention (ER) required for ME_m calculations (Whittemore, 1976; Böhme et al, 1980; Wenk et al, 1980; Noblet and Le Dividich, 1982; Campbell and Dunkin, 1983; Close and Stanier, 1984; McNutt and Ewan, 1984; Gadeken et al, 1985; Noblet et al, 1985).

Noblet et al. (1985) calculated the ME intake required for zero ER by linear regression. This regression method was previously reported by Halter et al (1980). Lodge et al (1979) proposed the following formulas to calculate ME_m:

If ER > 0, ME_m = intake – 1.43 * ER / (final wt – initital wt)/2

If ER < 0, ME_m = intake – 0.80 * ER / (final wt – initital wt)/2

The efficiency of energy utilization is accounted for in the formulas. In the first equation, 1.43 is the reciprocal of the efficiency of energy utilization from dietary sources of 0.70 previously reported by Verstegen et al. (1971). This equation is used when the ER is greater than zero, indicating energy intake greater than requirements. The second equation incorporates the value of 0.80 as this is the assumed efficiency of energy utilization from body tissue stores (Verstegen et al, 1971).

The ER is equal to the amount of protein and fat accretion multiplied by the corresponding energy contents of protein and fat (ARC, 1981). The ME_m is the energy required for "the non-productive basic energy needs of an animal" (Wenk et al, 1980). The nutrient needs of obligatory losses and vital processes "are just met" such that the net gain or loss of nutrients is zero (ARC, 1981).

To extrapolate from intakes greater than ME_m, ER is assumed to be zero at the ME_m energy intake (Noblet et al, 1985). However, the plane of nutrition has an effect on an animal's metabolic rate and therefore upon ER, HP, and calculated ME_m. As reported by Gray and McCracken (1980), ME_m values can only be properly determined when the animal is consuming approximately the ME_m energy intake. At greater than ME_m energy intake, the body metabolism adjusts to the greater energy intake and efficiency of energy use changes (decreases). Therefore, experiments should be designed to measure values for ME_m at a plane of nutrition as near to ME_m energy intake as possible.

1.13 Energy value of diets

Figure 1.1 Energy partition of food energy

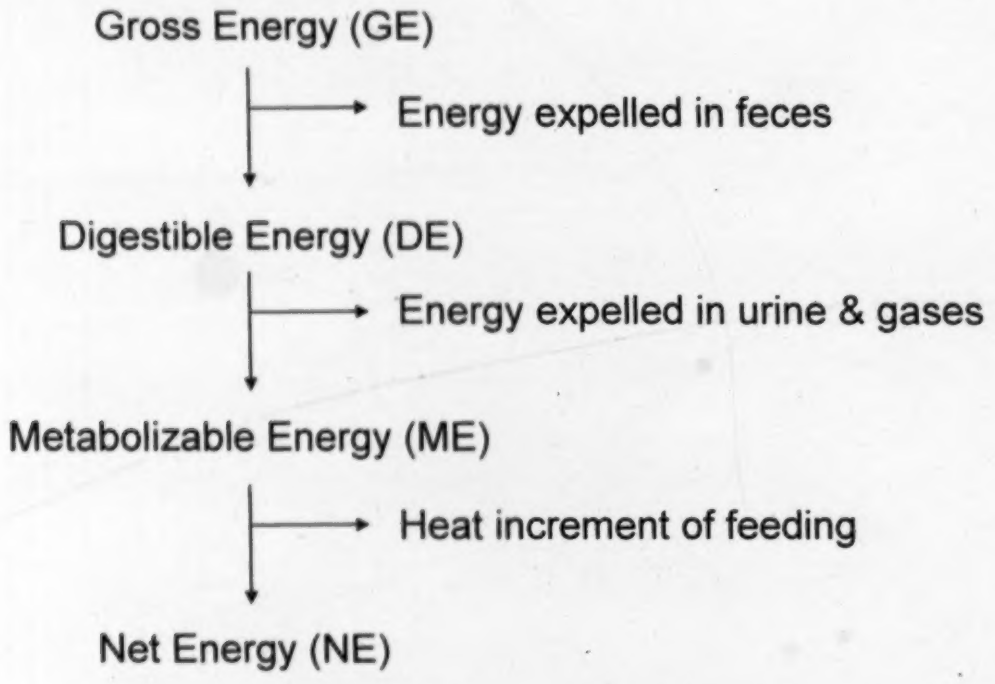


Figure 1.1 shows the partitioning of food energy from GE to NE. The GE of the diet is amount of energy released when the diet is burned, as determined by bomb calorimetry. However, the GE content of the diet does not in any way predict the amount of energy available to the animals. The classic example of this fact is that fibre, which is highly non-digestible by monogastric animals, may have the same GE as starch, but there is considerable difference in the energy digestibility (Boisen and Verstegen, 1998) because mammals lack β-1,4-glycosidase (E.C. 3.2.1.21) (Campbell, 1996). This leads to the next level of expressing energy contents of diets, known as Digestible Energy (DE). The DE is based on experimental evidence on the digestibility of the diets by swine. The effective difference between GE and DE is the amount of

energy lost in the feces of the animals. Energy losses in the form of skin and hair contribute to a negligible overestimate of energy lost in feces. Digested energy is subject to further metabolism and therefore losses. The energy retained by the body, less the energy lost in urine (i.e. urea, uric acid, creatine and creatinine) and as combustible gases (e.g. CH_4, H_2) is termed the metabolizable energy (ME). Because energy is required by the body for metabolic processes, thus leading to further losses of energy as heat, net energy (NE) reports the energy actually retained by the body via the deposition of body tissues and used for maintenance. The difference between ME and NE is energy lost to the environment as heat and is termed the heat increment of feeding (HIF). Various other losses of energy occur, including losses of skin and hair, but have little effect on the measurements of the energy value of diets.

The evolution of the different ways to express the energy content of food has a long history of research. The concept of NE dates as far back as 1922 when Armsby measured the NE value of timothy hay fed to steers (Kleiber, 1987). Only the most recent NRC (1998) contains any references to NE value of feeds for swine (Ewan, 1989; Noblet et al. 1994). Previously, energy contents of feeds or feedstuffs were expressed as DE or ME (NRC, 1979; NRC, 1988; Noblet et al. 1994). Thus, the NE concept has only recently been applied to swine nutrition in Europe (Noblet, 2007) while the adoption by nutritionists in North America continues to be delayed (Payne and Zijlstra, 2007). de Lange and Birkett (2005) and Patience and Beaulieu (2005) have reviewed the reasons for the delay in adoption of the NE system; specifically the lack of data about the energy contents of North American feedstuffs and feeds and the application across various body weight ranges and conditions. Therefore, further

research, including effects of feeding frequency (see 1.14), is required to increase the applicability of NE.

1.14 Effect of feeding frequency on energy metabolism

A number of reports (Leveille and Hanson, 1965; Han, 1973; Ozelci et al, 1977) have shown that rats trained to eat meals at specific times are able to gain weight as efficiently as ad lib fed rats. The trained rats usually eat 60% to 80% as much food as the ad lib fed rats. Allee et al. (1972) observed that pigs being fed a single meal consumed less food per day but gained weight at a rate similar to pigs fed frequent small meals. These reports suggest that there is greater efficiency of energy storage as feeding frequency decreases, as shown by Fábry (1969). This improvement in efficiency with which dietary energy is retained has also been attributed to reduced energy expenditure related to consuming a single meal compared to multiple meals. Baird (1970) found no advantage to feeding sows more than once per day with respect to the number of pigs born, born alive, strength of the piglets, or daily feed cost per sow.

According to Bellisle et al (1997) and Moehn et al (2004), feeding frequency does not have an effect on the associated HIF. It may, however, have an effect on the composition of the gain where reduced meal frequency sharply increases the rate of glucose "conversion to fatty acid . . . by adipose tissue" (Leveille and Hanson, 1966). In sows, eating a meal, regardless of size or frequency, will lead to greater energy expenditure at the time of feeding than during the fasting or postprandial state. This greater energy expenditure is a result of the associated production of enzymes and

increases in digestive processes. Greater energy expenditure during the postprandial state of frequently fed sows than of the meal fed sows indicates the continuation of digestion (i.e. HIF) (Moehn et al, 2004). As a result, there is a continuation of the short-term effect of feeding, as previously reported by Ramonet et al (2000), thus explaining greater postprandial energy expenditure in frequently fed sows. However, single meal feeding often reduces physical activity and, therefore, energy expenditure.

Reducing feeding frequency has an effect on the overall metabolism of an organism. Of particular note, is the influence of feeding frequency on lipid metabolism. As an adaptive mechanism for storage of large energy intakes, lipogenesis is stimulated by infrequent feeding (Leveille and Hanson, 1965). The result of increased lipogenesis is increased body fat and plasma lipid concentrations (Fábry, 1969). Although not reported by Ozelci et al. (1977) or Allee et al. (1972), it can be predicted that the composition of the gain between frequently fed and single fed animals would be quite different. Single fed animals retain more energy as fat and oxidize more amino acids due to the inability to deal with the large influx of nutrients (Fábry, 1969).

Feeding frequency has an effect on the composition of weight gain and efficiency of energy storage. Single meal fed animals tend to retain excess energy primarily as fat whereas frequently fed animals tend to store excess carbohydrates as glycogen, rather than converting them to lipid (Leveille and Hanson, 1965). Increasing the frequency of feeding may have an impact on the daily HP of swine. Therefore, it needs to be determined what, if any impact there is, and, if necessary, how to correct for this effect.

1.15 Respiratory quotient

Respiration experiments allow calculation of the respiratory quotient (RQ) because volumes of carbon dioxide produced and oxygen consumed are recorded over the course of the experiment. The RQ is calculated as the volume of CO_2 (V_{CO2}) divided by the volume of O_2 (V_{O2}) (i.e. V_{CO2} / V_{O2}). Values of RQ near 1.0 indicate carbohydrate oxidation because the volume of O_2 required for complete oxidation is equivalent to the amount of CO_2 produced due to the carbon to oxygen ratio in the carbohydrates. A RQ near 0.7 indicates fat oxidation because the carbon to oxygen ratio of the molecules highly favours carbon, so, as a result, more oxygen is required for complete oxidation.

The information given by the RQ about the substrate disappearance can be very useful in studies of energy metabolism (Simonson and DeFronzo, 1990). Generally, a RQ greater than 1 indicates lipogenesis (Chwalibog and Thorbek, 2000). Specifically, a RQ value of 5.6 indicates lipogenesis from glucose.. Therefore, RQ values greater than 1 indicate simultaneous lipogenesis and glucose oxidation (Ferrannini, E. 1988). Calculation of the RQ based on respiration data (i.e. volumes of gas exchange) alone is inaccurate because protein can also be oxidized for energy. A RQ value of 0.80 was calculated by Simonson and DeFronzo (1990) from animal muscle combustion. Calculating the RQ corrected for protein oxidation as measured by urinary nitrogen excretion equates to the non-protein RQ (NPRQ). However, according to Weir (1949), the effect of ignoring the urinary nitrogen (i.e. protein metabolism) is 1% for every 12.3% of the total dietary energy that was derived from protein.

Lipogenesis is common for sows because there is a nutritional goal toward a certain level of backfat (Aherne et al, 1999). Therefore, it is expected that RQ values will be greater than 1 when sows are fed excess energy. This is especially likely when sows are fed once per day (or adapted to being fed once per day) according to the common industry practice.

1.16 Adaptation to new energy intake

There are a number of factors affecting energy utilization and efficiency including feeding frequency (Allee et al, 1972) and feeding level (Noblet and Close, 1980; Kemp et al, 1987). These factors must be controlled in order to accurately determine the energy requirements and metabolism of animals. In order to standardize the experimental conditions, it may become necessary to impose strict eating regimens on the experimental animals. Therefore, the animals will require adaptation to the experimental eating regimens.

In the experiment of Gray and McCracken (1980), growing pigs were fed approximately three times ME_m and then their feed intakes were reduced to approximately the ME_m energy intake. On day zero, when feed intake was not yet reduced, the average HP was 1355 $kJ/kg^{0.56}$ and the RQ was 1.20. A RQ above 1 indicates the occurrence of lipogenesis and should be expected as the energy intake was greater than ME_m. On day one, when the feed intake was reduced to approximately the ME_m, the HP was lower and the RQ was reduced to 1.00. On day two, HP reduced and, interestingly, the RQ dropped to its lowest level during this study. The low RQ indicated that oxidation of body fat reserves was occurring, thus allowing the animal to

maintain a higher metabolic rate, even though feed intake had been reduced. After day three, a plateau of the HP and RQ values occurred indicating an adjustment of the metabolic rate of the animals' to the new energy intake. The RQ on days three to five was only slightly less than 1.0 (0.97 ± 0.01; CV<1%), indicating mostly dietary carbohydrate and some dietary lipid was metabolized for energy (i.e. energy metabolism only from dietary sources). On days three to five, HP (875.7 ± 17.2 $kJ/kg^{0.56}$; CV<2%) also stabilized. The plateau in RQ and HP indicated that metabolism was stable when energy intake was close to ME_m.

1.17 Improved productivity of modern, high producing sows

Domestic swine are raised to produce pork for human consumption. Originally, sows and their litters were housed and raised outdoors (Thornton, 1973). In many cases, people kept pigs in their yards for their ability to convert kitchen scraps and homegrown vegetables into meat and lard (Thornton, 1988). Modern pork production efficiency has been improved due to housing and genetics and their effects on sow productivity.

The first widely recognized management system for large-scale outdoor pork production was the Roadnight system. In this system, sows were farrowed twice per year in March and September with weaning occurring naturally 8 to 10 weeks later (Thornton, 1988). Modern pork production systems are housed entirely indoors with continuous farrowing. Indoor pork production has served to improve the productivity of sows by removing the effects of changing environment on the sows and their litters. Productivity has also been increased due to continuous farrowing and weaning at 3 to 4 weeks of age.

While sow productivity has been partially increased due to changes in management, the greatest changes have occurred more recently due to selective breeding for litter size and mothering ability (Figure 1.2 and Table 1.2). Merks (2000) reported that "improved knowledge of genetics increased genetic changes in . . . fertility and vitality" of modern swine. Modern sows produce and rear more piglets per litter and per year than even 10 years ago (Table 1.2).

Figure 1.2 Phenotypic trends in Canadian Yorkshire and Landrace populations

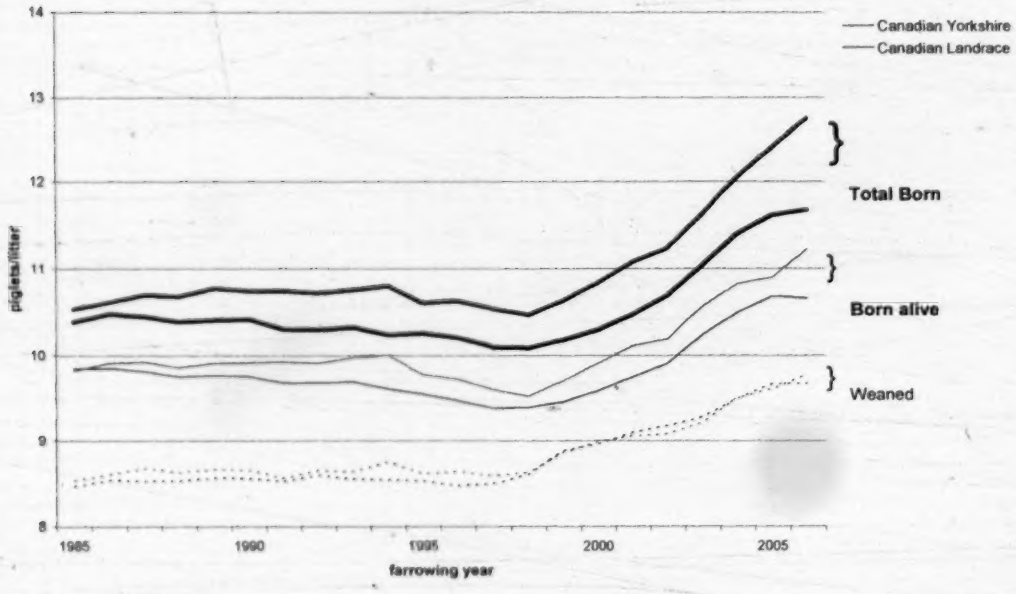


Table 1.2 Comparison of sow productivity – 1998 & 2004

Characteristic	In 1998 [a]	In 2004 [b]
Repeat services, %	10.7	9.2
Farrowing rate	75.6	84.3
Average non-productive days	61.2	56.2 [c]
Piglets born per litter	11.4	12.1
Litters per year	2.31	2.33
Piglets born per year	26.33	28.19
Piglets weaned per year	21.48	22.53
Age of litter at weaning (d)	21.3	20.43
Weight of litter at weaning (kg)	60	62.46
Days from weaning to re-breeding	6.7	7.5 [c]

[a] PigChamp Datashare 1998 - Annual data for Canada, n = 213
[b] PigChamp Datashare 2004 - Annual data for Canada, n = 39
[c] PigChamp Datashare 2003 - Annual data for Canada, n = 61
(http://www.pigchamp.com/summary_archives.html)

1.18 Summary

Modern sows have more piglets born per litter, rear more and heavier piglets to weaning, have more lactations per year, re-breed faster and more consistently, and are larger and leaner. Nutrient recommendations based upon research with sows that were much less prolific and productive than current sows should be suspect. Intense genetic selection has changed the basal metabolism of pigs. Thus the fundamental data underlying the current models for sow feeding must be updated.

1.19 Literature Cited

Agricultural Research Council. 1981. The nutrient requirements of pigs. Slough, U.K, Commonwealth.

Allee, G. L., D. R. Romsos, G. A. Leveille, & D. H. Baker. 1972. Metabolic adaptation induced by meal-eating in the pig. J. Nutr. 102: 1115-1122.

Arch, J. R. S., D. Hislop, S. J. Y. Wang, & J. R. Speakman. 2006. Some mathematical and technical issues in the measurement and interpretation of open-circuit indirect calorimetry in small animals. Int. J. Obesity. 30: 1322-1331.

Avram, A. S., M. M. Avram, & W. D. James. 2005a. Subcutaneous fat in normal and diseased states: 2. anatomy and physiology of white and brown adipose tissue. J. Am. Acad. Dermatol. 53: 671-683.

Avram, M. M., A. S. Avram, & W. D. James. 2005b. Subcutaneous fat in normal and diseased states: 1. introduction. J. Am. Acad. Dermatol. 53: 663-670.

Baird, D. M. 1970. Individual and "skip-day" vs. group feeding systems for sows during gestation. Research Report 82 ed. College of Agriculture Experiment Stations, University of Georgia.

Baldwin, R. L., & N. E. Smith. 1974. Molecular Control of Energy Metabolism. Pages 17-34 in The Control of Metabolism. Sink, J. D., ed. The Pennsylvania State University Press, University Park.

Blaxter, K. L. 1979. Discussion Paper: Use of Energy for Maintenance and Growth. Pages 183-187 in Energy Metabolism. Mount, L. E., ed. Butterworths, London.

Blaxter, K. L. 1971. Methods of measuring the energy metabolism of animals and interpretation of results obtained. Fed. Proc. 30(4): 1436-1443.

Böhme, H., D. Gädeken, & H. J. Oslage. 1980. Studies on energy costs of protein and fat deposition in early weaned piglets. Landw. Forsch. 33(2-3): 261-271.

Brody, S. B. Bioenergetics and growth. . New York, NY, Reinhold Pub. Corp.

Brouwer, E. 1965. Report of Sub-Committee on Constants and Factors. Pages 441-443 in Proceedings of the 3rd Symposium on Energy Metabolism. Blaxter, K. L., ed. 11th ed. EAAP Publication, Academic Press, New York, NY.

Brown-Brandl, T. M., J. A. Nienaber, H. Xin, & R. S. Gates. 2004. A literature review of swine heat production. T. ASAE. 47(1): 259-270.

Campbell, N. A. 1996. Biology. 4th ed. Menlo Park, California, The Benjamin/Cummings Publishing Company, Inc.

Campbell, R. G., & A. C. Dunkin. 1983. The effects of energy intake and dietary protein on nitrogen retention, growth performance, body composition and some aspects of energy metabolism of baby pigs. Br. J. Nutr. 49: 221-230.

Christensen, K., A. Chwalibog, S. Henckel, & G. Thorbek. 1988. Heat production in growing pigs calculated according to the RQ and CN methods. Comp. Biochem. Physiol. 91A(3): 463-468.

Chwalibog, A., & G. Thorbek. 2000. Estimation of net nutrient oxidation and lipogenesis in growing pigs. Arch. Anim. Nutr. 53: 253-271.

Close, W. H., & M. W. Stanier. 1984. Effects of plane of nutrition and environmental temperature on the growth and development of the early-weaned piglet. 2. Energy metabolism. Anim. Prod. 38: 221-231.

Fábry, P. 1969. Feeding pattern and nutritional adaptations. London, Butterworths.

Gädeken, D., H. J. Oslage, & H. Böhme. 1985. Energy requirement for maintenance and energy costs of protein and fat deposition in piglets. Arch. Tierernähr. 7: 481-494.

Garrett, R. H., & C. M. Grisham. 1999. Biochemistry. 2nd ed. Florida, USA, Saunders College Publishing.

Han, I. K. 1973. Effect of frequency of meal on growth rate, nutrient digestibility, body composition, nitrogen retention and heat production of rats. Nutr. Rep. Int. 7(1): 9-18.

Holmes, C. W., & K. Breirem. 1974. A note on the heat production of fasting pigs in the range 16 to 96 kg live weight. Anim. Prod. 18: 313-316.

Institute of Medicine. 2005. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington, D.C., National Academy Press.

Kelly, J. M., & B. W. McBride. 1990. The sodium pump and other mechanisms of thermogenesis in selected tissues. P. Nutr. Soc. 49: 185-202.

Kienzle, E., I. Schrag, R. Butterwick, & B. Opitz. 2001. Calculation of gross energy in pet foods: New data on heat combustion and fibre analysis in a selection of foods for dogs and cats. J. Anim. Physiol. 85: 148-157.

Kleiber, M. 1987. The fire of life: An introduction to animal energetics. 3rd ed. Florida, USA, Robert E. Krieger Publishing Co., Inc.

Laidler, K. J., & J. H. Meiser. 1999. Physical chemistry. 3rd ed. Boston, MA, Houghton Mifflin Company.

LeBlanc, J., & P. Diamond. 1986. Effect of meal size and frequency on postprandial thermogenesis in dogs. Am. J. Physiol. 250: E144-E147.

Leveille, G. A., & R. W. Hanson. 1966. Adaptive changes in enzyme activity and metabolic pathways in adipose tissue from meal-fed rats. J. Lipid Res. 7: 46-55.

Leveille, G. A., & R. W. Hanson. 1965. Influence of periodicity of eating on adipose tissue metabolism in the rat. Can. J. Physiol. Pharmacol. 43(6): 857-868.

Lipmann, F. 1941. Metabolic Generation and Utilization of Phosphate Bond Energy. Pages 99-162 in Advances in Enzymology and Related Subjects of Biochemistry. 1st ed. John Wiley & Sons Inc., New Jersey, USA.

Lodge, G. A., D. W. Friend, & M. S. Wolynetz. 1979. Effect of pregnancy on body composition and energy balance of the gilt. Can. J. Anim. Sci. 59: 51-61.

McNutt, S. D., & R. C. Ewan. 1984. Energy utilization of weanling pigs raised under pen conditions. J. Anim. Sci. 59(3): 738-745.

Merks, J. W. M. 2000. One Century of Genetic Changes in Pigs and the Future Needs. Pages 8-19 in The Challenge of Genetic Change in Animal Production. Hill, W. G., S. C. Bishop, B. McGuirk, J. C. McKay, G. Simon & A. J. Webb, eds. 27th ed. Occ. Publi. Br. Soc. Anim. Sci., BSAS, Edinburgh, UK.

Milligan, L. P. 1971. Energetic efficiency and metabolic transformations. Fed. Proc. 30(4): 1454-1458.

Milligan, L. P., & M. Summers. 1986. The biological basis of maintenance and its relevance to assessing responses to nutrients. P. Nutr. Soc. 45: 185-193.

Moehn, S., R. F. P. Bertolo, P. B. Pencharz, & R. O. Ball. 2004. Pattern of carbon dioxide production and retention is similar in adult pigs when fen hourly, but not when fed a single meal. BMC Physiology. 4: 11-18.

National Research Council. 1998. Nutrient requirements of swine. 10th ed. Washington, D.C., National Academy Press.

Noblet, J., C. Karege, & S. Dubois. 1989. Influence of Sex and Genotype on Energy Utilization in Growing Pigs. Pages 57-60 in Energy Metabolism of Farm Animals. van der Honing, Y., & W. H. Close, eds. 43rd ed. EAAP Publication, Wageningen Pers.

Noblet, J., & J. Le Dividich. 1982. Effect of environmental temperature and feeding level on energy balance traits of early-weaned piglets. Livest. Prod. Sci. 9: 619-632.

Noblet, J., J. Le Dividich, & T. Bikawa. 1985. Interaction between energy level in the diet and environmental temperature on the utilization of energy in growing pigs. J. Anim. Sci. 61(2): 452-459.

Ozelci, A., D. R. Romsos, & G. A. Leveille. 1977. Influence of diet composition on nitrogen balance and body composition in meal-eating and nibbling rats. J. Nutr. 107: 1768-1774.

Ramonet, Y., J. van Milgen, J. Y. Dourmad, S. Dubois, M. C. Meunier-Salaun, & J. Noblet. 2000. The effect of dietary fibre on energy utilisation and partitioning of heat production over pregnancy in sows. Br. J. Nutr. 84(1): 85-94.

Rolfe, D. F. S., & G. C. Brown. 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol. Rev. 77(3): 731-758.

Salway, J. 1999. Metabolism at a glance. 2nd ed.Blackwell Science Ltd.

Simonson, D. C., & R. A. DeFronzo. 1990. Indirect calorimetry: Methodological and interpretative problems. Am. J. Physiol. 258: E399-E412.

Soucy, J., & J. LeBlanc. 1999. Protein meals and postprandial thermogenesis. Physiol. Behav. 65: 705-709.

Tess, M. W., G. E. Dickerson, J. A. Nienaber, & C. L. Ferrell. 1984a. The effects of body composition on fasting heat production in pigs. J. Anim. Sci. 58(1): 99-110.

Tess, M. W., G. E. Dickerson, J. A. Nienaber, J. T. Yen, & C. L. Ferrell. 1984b. Energy costs of protein and fat deposition in pigs fed ad libitum. J. Anim. Sci. 58(1): 111-122.

Thorbek, G., A. Chwalibog, & S. Henckel. 1984. Nitrogen and energy metabolism in pigs of danish landrace from 20 to 120 kg live weight. Beretning Fra Statens Husdyrbrugs Forsøg. 563: 114.

Thorbek, G., A. Chwalibog, & S. Henckel. Nitrogen and energy metabolism in pigs of danish landrace from 20 to 120 kg live weight. Beretning Fra Statens Husdyrbrugs Forsøg. 563: 114.

van Milgen, J., J. Noblet, S. Dubois, & J. -F. Bernier. 1997. Dynamic aspects of oxygen consumption and carbon dioxide production in swine. Br. J. Nutr. 78: 397-410.

Weir, J. B. d. 1949. New methods for calculating metabolic rate with special reference to protein metabolism. J. Physiol. 109: 1-9.

Wenk, C., P. C. Colombani, J. van Milgen, & A. Lemme. 2001. Glossary: Terminology in Animal and Human Energy Metabolism. Pages 409-421 103rd ed. EAAP Publication, Wageningen Pers.

Wenk, C., H. P. Pfirter, & H. Bickel. 1980. Energetic aspects of feed conversion in growing pigs. Livest. Prod. Sci. 7: 483-495.

West, G. B., J. H. Brown, & B. J. Enquist. 2000. The Origin of Universal Scaling Laws in Biology. Pages 87-112 in Scaling in Biology. Brown, J. H., & G. B. West, eds. Oxford University Press, New York.

White, C. R., & R. S. Seymour. 2005. Allometric scaling of mammalian metabolism. J. Exp. Biol. 208: 1611-1619.

Whittemore, C. T. 1976. A study of growth responses to nutrient inputs by modelling. P. Nutr. Soc. 35: 383-391.

2.0 RATIONALE, HYPOTHESES AND OBJECTIVES

2.1 Rationale

Feed represents 60 to 70% of the total cost of pork production. Within this fraction, the greatest cost is the provision of energy (Noblet and Henry, 1993). The next most expensive and important feed ingredient after energy is protein. Protein is provided as intact proteins and/or individual, synthetic amino acids. Therefore, to produce pork as cost effectively as possible, the amount of energy and protein both required by the animal and provided by the diet must be accurately known.

Targeted delivery of energy and protein is two-pronged: 1) the provision of energy and protein can be focused to allow optimum growth (nursery, growers) and production (sows), or 2) energy and protein intakes can be controlled to produce uniform pork products by regulating carcass characteristics (finishers). The central importance of energy and protein in pork production has led to considerable research, as summarized by NRC (1998). However, the majority of the research has been focused on growing pigs and relatively little research has been on sows.

Sows have central importance to pork production because they are the reproductive unit of the swine herd. However, sows represent a numerically small fraction of the total pig herd and consume 20% of the feed for pork production. Sow feeds have progressed from the days of outdoor grazing supplemented with kitchen scraps (Thornton, 1988) to the current complex feed mixtures of specific ingredients fed to completely indoor pig herds (Thornton, 1973). The supplied, mixed rations are the only feed source for indoor pork production, therefore, it is critical that the nutritionist

formulate the diets to meet the dietary requirements of the animals as closely as possible.

Improper diets have the potential to cause negative effects on body composition, including loss of backfat due to energy restriction and loss of body protein due to amino acid limitations, leading to reduced longevity, poor rebreeding success and capacity, and lower litter birth and weaning weights. NRC (1998) reports that for sows "during gestation, 60 to 80 percent of the total energy requirement is used for maintenance". Therefore, limitations in energy intake will significantly impact the body composition of the sow, especially given that sows will mobilize body reserves to maintain healthy growth of the litter (Walach-Janiak et al, 1986).

Gestation is the most common physiological state of reproducing sows, being of three months duration versus three weeks of lactation. However, the impact of lactation on the maternal body reserves is much greater than any other healthy physiological state (Baracos, 2006). Evidence exists showing that there is an impact of the previous lactation on the subsequent reproductive performance (Baidoo et al, 1992; Hazeleger et al, 2005; Clowes et al, 2003a,b; Anil et al, 2006).

Current feeding recommendations (NRC 1998; Aherne et al, 1999) are not appropriate for optimum productivity of the modern, high producing sow. Modern breeds are a result of constant genetic selection for greater growth rates and higher productive and reproductive capacity (Foxcroft et al, 2008). Significant increases in the growth rates of piglets coupled with increased litter sizes has increased the energy required to support maximum milk production. Decreased body tissue energy reserves

of modern sows, mostly as backfat, requires that any increased demand for energy be supplied by the diet, not by mobilization of maternal body tissue.

Studies of modern sows at maintenance will quantify the basal values of energy metabolism to which other energy requirements may be added. Therefore, it is necessary to study the energy metabolism of sows in the physiological states of gestation and lactation to quantify the energy required in excess of maintenance. Clearly, the need exists for improvements on the current dietary energy recommendations to reflect the needs of modern sows.

2.2 Null hypotheses

1) Body weight gain, diet digestibility, heat production, and respiratory quotient will not be different between sows fed at 2.0 times their maintenance energy requirement and sows fed at their maintenance energy requirement.
2) The heat increment of feeding, and therefore the NE content of the diet, will not be different between sows being fed frequent, small meals compared to sows fed a single large meal.
3) Body weight gain, diet digestibility, heat production, and respiratory quotient of gravid sows will not be different at early-, mid-, and late-gestation and will not vary with products of conception.

2.3 Specific objectives

1) 24 hour heat production will be measured to determine the energy expenditure of sows fed 1.0 and 2.0 times their maintenance energy requirement and a comparison to previously reported maintenance energy requirement values will be made.
2) 24 hour heat production will be measured for non-pregnant sows as they are fed 1/32 of their daily ration each 30 minutes for 8 hours before receiving a single meal of ½ of their daily ration to compare the heat increment of feeding and NE content of the diet for nibbling and single meal feeding between sows fed 1.0 and 2.0 times their maintenance energy requirement.
3) Nutrient and energy digestibility and body weight gain will be measured for non-pregnant sows fed 1.0 and 2.0 times their maintenance energy requirement.
4) Nutrient and energy digestibility, body weight gain, and body composition changes will be measured on days 30, 45, and 105 of gestation and compared to the NRC (1998) factorial equations for body weight gain, energy retained and expended by the sow vs. the energy retained and expended by the products of conception. New factors will be proposed, if required.
5) The respiratory quotient within and between experiments will be compared for differences in substrate utilization using the average RQ from similar feeding frequencies.

2.4 Literature Cited

Aherne, F. X., G. R. Foxcroft, & J. E. Pettigrew. 1999. Nutrition of the Sow. Pages 1029-1044 in Diseases in Swine. Straw, B. E., S. D'Allaire, W. L. Mengeling & D. J. Taylor, eds. 8th ed. Iowa University Press, Ames, Iowa.

Anil, S. S., L. Anil, J. Deen, S. K. Baidoo, & R. D. Walker. 2006. Association of inadequate feed intake during lactation with removal of sows from the breeding herd. J. Swine Health Prod. 14: 296-301.

Baidoo, S. K., F. X. Aherne, R. N. Kirkwood, & G. R. Foxcroft. 1992. Effect of feed intake during lactation and after weaning on sow reproductive performance. Can. J. Anim. Sci. 72: 911-917.

Ball, R. O., R. S. Samuel, & S. Moehn. 2008. Nutrient requirements of prolific sows. Advances in Pork Production. 19: 223-236.

Baracos, V. 2006. Integration of amino acid metabolism during intense lactation. Curr. Opin. Clin. Nutr. 9: 48-52.

Clowes, E. J., F. X. Aherne, G. R. Foxcroft, & V. E. Baracos. 2003. Selective protein loss in lactating sows is associated with reduced litter growth and ovarian function. J. Anim. Sci. 81: 753-764.

Clowes, E. J., F. X. Aherne, A. L. Schaefer, G. R. Foxcroft, & V. E. Baracos. 2003. Parturition body size and body protein loss during lactation influence performance during lactation and ovarian function at weaning in first-parity sows. J. Anim. Sci. 81: 1517-1528.

Foxcroft, G. R. 2008. Hyper-prolificacy and acceptable post-natal development - a possible contradiction. Advances in Pork Production. 19: 205-211.

Hazeleger, W., N. M. Soede, & B. Kemp. 2005. The effect of feeding strategy during the pre-follicular phase on subsequent follicular development in the pig. Domest. Anim. Endocrin. 29: 362-370.

National Research Council. 1998. Nutrient requirements of swine. 10th ed. Washington, D.C., National Academy Press.

Noblet, J., & Y. Henry. 1993. Energy evaluation systems for pig diets: A review. Livest. Prod. Sci. 36: 121-141.

Thornton, K. 1988. Outdoor pig production. 1st ed. Ipswich, Suffolk, Farming Press Ltd.

Thornton, K. 1973. Practical pig production. 1st ed. Ipswich, Suffolk, Farming Press Ltd.

Walach-Janiak, M., S. Raj, & H. Fandrejewski. 1986. Protein and energy balance in pregnant gilts. Livest. Prod. Sci. 15: 249-260.

3.0 THE 24 h HEAT PRODUCTION OF NON-PREGNANT SOWS FED AT 473 kJ/BW$^{0.75}$ AND 925 kJ/BW$^{0.75}$

3.1 Introduction

Feed intake of sows is controlled by the production manager in modern pork production systems. Feed allowance is based on the energy available in the mixed ration, sow body condition, and the reproductive status of the animals in the production system. Energy intake targets for swine are based on the current estimates of the metabolizable energy (ME) for maintenance requirement (ME_m) for swine (ARC, 1981; NRC, 1998).

Sows have changed a great deal in the recent past, including: body composition, growth rate, adult body weight, reproductive capacity, re-breeding success, and longevity (Wenk et al, 1980; Ball et al, 2008). Differences in these individual factors have coupled together resulting in very different sows than even a few years ago. Selective breeding has reduced the whole-body fat content of modern sows while increasing the lean-tissue content. Also the average body weight and size of sows has increased. As a result, the heat production (HP) of modern sows is predicted to have increased by at least 18% (Brown-Brandl et al, 2004).

Measurement of daily (24 h) HP or comparative slaughter can be used to calculate energy retention (ER) and, subsequently, the daily ME_m. While both methods are relevant; measurement of HP by gas exchange additionally allows calculation of the respiratory quotient, which indicates the proportion of energy derived from different dietary substrates. Measurement of HP can also be used to elucidate changes in body

composition. A greater number of animals are required to determine the ER by comparative slaughter due to the errors involved in the analysis (Kempster et al, 1982). All animals have to be killed, so repeated measurements under differing conditions are not possible, and the measurements cannot be individualized as they can be with calorimetry techniques. Therefore, measurement of HP provides greater information and is therefore preferable to slaughter techniques.

According to Wenk et al (1980) HP for determining the ME_m should be measured as close to zero ER as possible, but some researchers have estimated ME_m when energy intakes were much greater than ME_m. In addition when ME_m is measured, sows must be non-pregnant because pregnancy imposes significant additional metabolic demands in addition to maintenance.

Recommended values for the ME_m of swine on a per day basis are 458 kJ ME/kg $BW^{0.75}$ (ARC, 1981) and 106 kcal ME/kg $BW^{0.75}$ (equivalent to 444 kJ ME/kJ $BW^{0.75}$) (NRC, 1998). By using these values and the ME content of the diet, energy requirements can be translated into daily dietary feed intakes. Calculation of daily feed intakes has become a standard practice for production managers to ensure the correct energy intakes for the sows. These calculations, and consequently proper energy intake, depend on correct and reliable estimates of maintenance energy requirements. However, current recommendations for sows were developed more than 10 years ago and are based on estimates of ME_m determined in piglets and growing pigs (NRC, 1998) or sows with very different body composition and reproductive performance than current sows (Lodge et al, 1979; Noblet and Close, 1980). Therefore, we hypothesized that current ME_m estimates are too low for modern, highly prolific sows.

The objective of this experiment was to determine and compare the 24 h HP of non-pregnant sows when fed two different energy intakes representing an estimate of ME_m and 2 times ME_m. In conjunction with energy and nutrient digestibility determinations and body weight changes, the ME_m of modern non-pregnant sows will be determined and compared between sows fed two different energy intakes. An additional goal of this experiment, and specific feeding schedule as designed, was to enable future comparison among results from studies of shorter duration (e.g. 6 or 8 h) to be extrapolated to 24 h measurements. The null hypothesis for this experiment was "body weight gain, diet digestibility, energy expenditure, and respiratory quotient will not be different between sows fed at 2.0 times the recommended maintenance energy requirement and sows fed the recommended maintenance energy requirement".

3.2 Materials and Methods

3.2.1 Animals and ethics approval

Sows (n=5, 174 kg ± 11 kg) were selected from the University of Alberta's Swine Research and Technology Centre (SRTC). Sows of similar body weight and parity were selected along with a visual assessment for similar and desirable conformation. All procedures were approved by the University of Alberta's Faculty Animal Policy and Welfare Committee and were in accordance with guidelines of the Canadian Council on Animal Care.

3.2.2 Diets, feeding, and housing

The diet was formulated and composed of readily available feedstuffs for Western Canada. The diet was the same in composition as the usual Dry Sow Ration used by the SRTC, as shown in Table 3.1. The same diet was fed during the whole experiment, but the amount offered was doubled when sows were fed 2x ME_m. The sows were fed 1x ME_m first and then 2x ME_m to avoid possible confounding factors on energy metabolism. The analyzed means and SEM of the diet as fed, shown in Table 3.2, represent pooled results from the proximate analysis of ground feed samples. Samples were collected at random following the addition and mixing of the indigestible marker (see 3.2.2.1). Sows were fed one-half of their daily feed allowance (see 3.2.2.2) twice daily at 09:00 and 15:00, except on study days (see 3.2.2.3). Individual nipple drinkers provided access to water at all times. Sows were housed individually in raised pens with plastic flooring. Pens were cleaned daily and washed frequently.

Table 3.1 Diet composition of dry sow ration

Ingredient	g/kg
Wheat[1]	121
Barley[2]	700
SBM[3]	65
Canola Meal[4]	65
Canola Oil	9
Breeder Premix[5]	40
Calculated	
Ca, %	0.95
Total P, %	0.72
DE, MJ/kg	13.06
CP, %	14.76
Total Lys, %	0.65
DigLys/CP	3.28
Na, %	0.22
Se, mg added	0.5
Vit A, IU	12000
Vit D, IU	1400
Vit E, IU	72

[1]: 12.5% CP; [2]: 11.2% CP; [3]: 48% CP; [4]: 35% CP; [5]: Provided per kilogram of the diet: Ca, 8.6g; P, 3.4g; Na, 1.9g; Mg, 140mg; K, 30mg; Fe, 139 mg; Zn, 119 mg; Mn, 56 mg; Cu, 16 mg; Co, 0.4 mg; I, 0.4 mg; Se, 0.3 mg; vitamin A, 12,000 IU; vitamin D_3, 1200 IU; vitamin E, 62 IU; vitamin K, 2.5mg; biotin, 0.6 mg; folic acid, 2.5mg; niacin, 42 mg; pantothenic acid, 25 mg; pyridoxine, 5mg; riboflavin, 9.5 mg; thiamine, 8.4mg; vitamin B_{12}, 28µg.

Table 3.2 Proximate analysis of the dry sow ration as-fed

	473 kJ/BW$^{0.75}$		925 kJ/BW$^{0.75}$			
Analyzed	**N**	**Mean**	**N**	**Mean**	**SEM**	**P-value**
GE, MJ/kg	3	15.97	3	16.18	0.07	<0.05
DE, MJ/kg	4	12.77	2	13.33	0.20	0.28
CP, %	3	16.02	3	15.91	0.13	0.57
Fat, %	3	2.47	3	2.09	0.04	<0.01
NDF, %	3	14.74	3	15.22	0.56	0.54
ADF, %	3	5.56	3	5.57	0.16	0.98
AIA, %	3	1.48	3	1.42	0.05	0.53
Ash, %	3	6.72	3	6.72	0.01	0.99

3.2.2.1 Addition of Celite® (acid insoluble ash)

Celite®, as an indigestible marker, was added and mixed into individual batches of complete SRTC Dry Sow Ration. Celite® was added at 10 g/kg of diet and analyzed as acid insoluble ash (AIA) (McCarthy et al, 1977).

3.2.2.2 Calculation of daily feed allowance

The daily feed intake of the sows was controlled assuming a maintenance energy requirement (ME_m) of 458 kJ ME/$kg^{0.75}$ (ARC, 1981). Sows were fed the SRTC Dry Sow Ration for ME_m intake for an adaptation period of 7 days until following the first respiration experiment. During the second adaptation period of 7 days and respiration experiment, sows were fed the SRTC Dry Sow Ration to achieve twice ME_m intake.

3.2.2.3 Experimental feeding regimen

On respiration days, sows were fed after they were secured into a respiration chamber. Sows received meals according to the following schedule (Figures 3.1 and 3.2):

1) an initial meal of $1/16^{th}$ of their daily ration immediately after entering the respiration chamber,
2) meals of $1/32^{nd}$ of their daily ration every 30 minutes afterward, and
3) a single meal of ½ of their daily ration 30 minutes after the last small meal was offered.

Figure 3.1 Graphical representation of the data collection 'period' by experimental feeding regimen

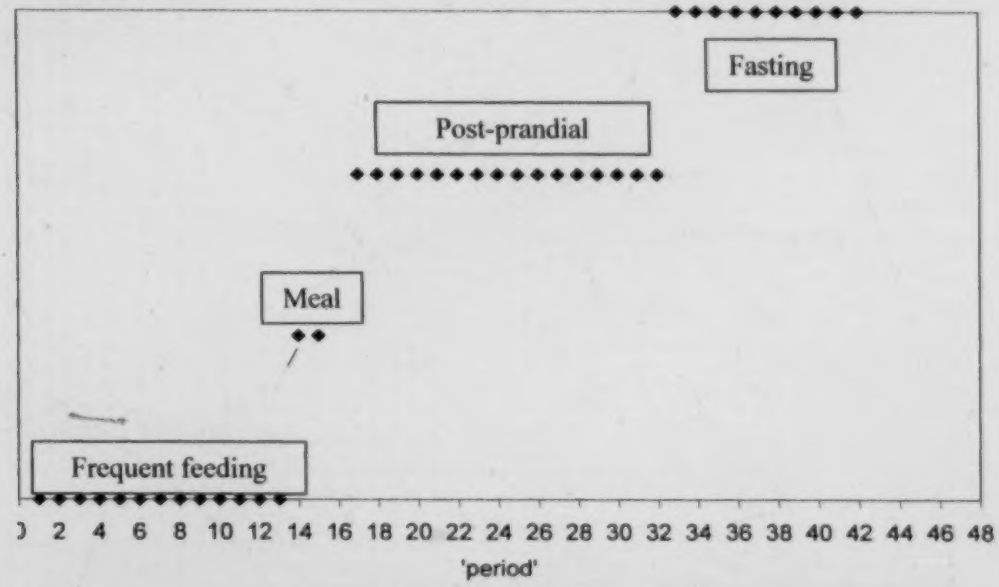
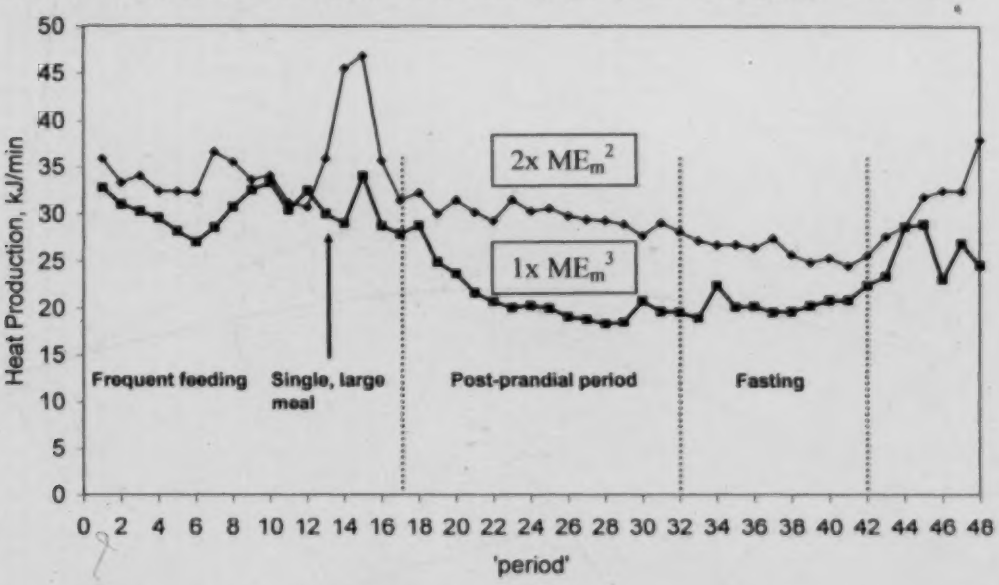


Figure 3.2 Mean heat production of sows fed 473 kJ/BW$^{0.75}$ (1x ME$_m$) versus 925 kJ/BW$^{0.75}$ (2x ME$_m$) according to collection 'period' and feeding regimen 'states'[1]



[1]: frequent feeding = 1/32 of daily feed allowance every 30 minutes; single, large meal = ½ of daily feed allowance after 8 h; post-prandial = starting 2 h after large meal and for 8 h; fasting = 10 h after large meal and for 6 h; [2]: 925 kJ/BW$^{0.75}$; [3]: 473 kJ/BW$^{0.75}$

3.2.3 Body weight and daily gain

Sows were weighed individually, after an overnight fast, to determine their feed allowance before the start of the adaptation periods and again on respiration days. The scale head rounded to the nearest 200 g and included an averaging feature to improve the accuracy of the measurements. Daily gain was calculated for each individual animal.

3.2.4 Indirect calorimetry[5]

Before each study, the analyzers (Qubit Systems, Kingston, Ontario) were calibrated to zero with N_2 for the O_2 and CO_2 analyzers and a gas of known $[O_2]$ and the balance N_2 for the CH_4 analyzers. The upper limits of the analyzers were calibrated with a gas of known $[O_2]$, $[CO_2]$, and $[CH_4]$ and the balance N_2 (Praxair, Edmonton, Alberta). O_2, CO_2, and CH_4 concentrations were collected by C409 data acquisition system as an average of 200 samples in one-minute intervals during the study (Qubit Systems, Kingston, Ontario). The gas exchange of the animals was determined by comparison to the ambient air values as recorded immediately before and after the study periods. The analytical values for each of the calibration gases was recorded immediately before and after the study and used to correct for drift of the analyzers, if necessary.

Two independent open-circuit indirect calorimetry chambers were each fitted with a 10cm diameter capped PVC tube which allowed feed to be dropped into the feeder and a nipple drinker for *ad libitum* fresh water intake. Access to the animals was through a clear plexiglass window on the top of each chamber. The calorimetry

[5] Further details can be found in the Appendix.

chambers were designed with two air inlets each consisting of 2.5 cm diameter ABS pipe the length of the chamber with holes drilled approximately every 30cm and capped at the opposite end. Ambient air was drawn into the chamber through these inlet pipes by vacuum displacement as air was withdrawn from the chamber. Air flow out of the chambers of 250 L/min was required to maintain [CO_2] below 1.0%. The animals were directed into the chambers through a rear door that was closed to isolate air flow to the inlet and outlet pipes. The volume of air from the outlet of each box was measured by separate AC630[6] gas meters and manually recorded every 30 minutes. A sub-sample (250 ml/min) from each chamber was dried over a column of drierite before being directed to the analyzers.

3.2.5 Chemical analyses

3.2.5.1 Sample grinding

Feed and freeze dried fecal samples were ground in a commercial coffee grinder before analysis to reduce particle size.

3.2.5.2 Bomb calorimetry

Approximately 1g ground samples were weighed in duplicate into tared metal cups. The cup was then placed into the holder in the top of the bomb (LECO Corporation, St. Joseph, Michigan). A 10cm length of platinum wire was situated the slits on the posts of the top of the bomb, just touching the sample in the cup. The top was put onto the bottom of bomb and the ring screwed down to seal the bomb. The

[6] Gas meters are calibrated annually to within 0.1% by Enertech Mechanical, Edmonton, Alberta.

bomb was charged with O_2 to 450 psi. The computer was previously readied by clearing old data. The weight of the current sample was entered, 'Enter' was pressed and the computer sequence began by lowering the bomb into the water chamber. After the sample in the bomb had been completely oxidized, the computer sequence ended and the bomb was raised from the water chamber. Excess O_2 was released from the bomb and the bomb was opened. After visually confirming complete oxidation of the sample, dH_2O with Methyl Orange indicator was used to wash the interior and top of the bomb into a beaker. The washing was complete when there was no longer a colour change of the rinse solution. The solution in the beaker was titrated with 0.8 *N* $Na_2CO_{3(aq)}$ to an orange endpoint. The volume of titrant and the length of wire burned (i.e. 10cm – length unburned) was entered and 'Enter' was pressed twice. The caloric content (cal/g) was then printed out by the computer.

3.2.5.3 Acid insoluble ash content

Approximately 2 g samples of ground feed in quadruplicate or 0.5 g samples in duplicate of ground, dried feces were weighed into weighed and labelled 175x12mm glass test tubes. The test tubes were held upright in a 600 mL Pyrex beaker then placed into a 500 °C oven overnight. The ashed samples were cooled to room temperature (RT) before 1.0 ml of 4 *N* $HCl_{(aq)}$ was added to each tube and vortexed. An additional 4 ml of 4 *N* $HCl_{(aq)}$ was then added to each tube. Marbles were placed on top of each of the tubes and heated at 120 °C overnight. After cooling to RT, the sample tubes were centrifuged at 3000 rpm for 10 minutes. The supernatant was removed by vacuum before 5 ml of ddH_2O was added to each tube and vortexed. The tubes were then

centrifuged at 3000 rpm for 10 minutes before the supernatant was again removed by vacuum. This washing step was repeated for a total of 3 times. After the final washing, the sample tubes were placed in a 90 ℃ oven overnight. Once the samples were completely dry, they were placed into a 500 ℃ oven for 24 hours. The ashed samples were removed and cooled to RT in a dessicator. Final weights of the tubes were recorded. Acid insoluble ash (AIA) content was calculated as:

$$\frac{\text{Final tube weight} - \text{Initial tube weight}}{\text{Sample weight}} \times 100 = \% \text{ AIA}$$

3.2.5.4 Crude fat content

Approximately 5 g of sample was weighed in duplicate into tared thimbles in metal holders. A small plug of glass wool was added to the top of each thimble. The metal holders were clipped into place on the Goldfisch fat extraction apparatus, previously switched on and with the condensing water running. Approximately 20 ml of petroleum ether was added to each pre-weighed Goldfisch apparatus beaker before being tightened into place by the metal ring. The heaters were turned on and each heater was raised to within 1 cm of the corresponding beaker. The system was checked for leaks of petroleum ether and, after rectifying any leaks, left to reflux for 4 hours. The heaters were lowered and the beakers allowed to cool. The beakers were removed and the sample holders replaced by glass cups. The heaters were again raised to evaporate the petroleum ether into the glass cups. When there was at least 3 mm of solution left in the beaker, the heaters were lowered and switched off. The beakers were left overnight in a fume hood to dry off the remaining petroleum ether. The dried beakers were then

placed in 110 °C oven for at least 3 hours before cooling in a dessicator. The cooled beakers were then weighed and the crude fat (CF) content determined as:

$$\frac{(\text{Final weight of beaker} - \text{Initial weight of beaker}) - \text{Blank beaker weight}}{\text{Sample weight}} \times 100 = \%CF$$

3.2.5.5 Neutral detergent fibre (NDF) content

Between 0.45 – 0.55 g of previously ground sample was weighed and heat-sealed in duplicate into sample bags (ANKOM). A maximum of twenty-four bags, including an empty, heat-sealed blank, were arranged in the trays of the apparatus. 1.9 L of neutral detergent fibre (NDF) solution (30.0 g sodium lauryl sulfate, USP; 18.61g Ethylenediaminetetraacetic disodium salt, dihydrate; 6.81 g sodium tetraborate decahydrate; 4.56 g sodium phosphate dibasic, anhydrous; and 10.0 ml triethylene glycol per 1 L distilled H_2O) was added on the top the bags held in the apparatus. 20 g of sodium sulfite and 4 ml of heat-stable alpha-amylase were also added. The top of the apparatus was closed and the heat and agitate were turned on. After 75 minutes, heat and agitate were turned off, the solution was drained, and then the lid was opened. After closing the drain valve, 1.9 L of hot water (80 °C) and 4 ml of alpha-amylase were added, agitated for 5 minutes and then drained. This rinse process was repeated a total of three times, omitting the alpha-amylase on the final rinse. The bags were removed from the apparatus, excess water was gently pressed out, and then soaked in acetone. After 5 minutes, the excess acetone was gently pressed out. The bags were allowed to dry at RT overnight and then at 110 °C for 4 hours before being cooled in specific dessicator bags (ANKOM) and weighed. The %NDF was calculated as:

$$\frac{\text{Final bag weight} - (\text{Initial bag weight} \times \text{Blank bag correction})}{\text{Sample weight}} \times 100$$

Where,

Blank bag correction = (Final Blank bag weight/Initial Blank bag weight)

3.2.5.6 Acid detergent fibre (ADF) content

Duplicate ground samples of 0.45 – 0.55 g were previously prepared for and run through the NDF procedure (see 3.3.6). Twenty-four bags, including one blank bag, were arranged in the trays and placed into the apparatus (ANKOM). 1.9 L of acid detergent fibre (ADF) solution (20 g cetyl trimethylammonium bromide (CTAB) in 1 L 1.00*N* H_2SO_4) was added to the apparatus on top of the samples bags. Heat and agitate were turned on. After 60 minutes, heat and agitate were turned off. The ADF solution was drained and the lid was opened. After closing the drain valve, approximately 1.9 L of hot water (80 °C) was added. After agitating for 5 minutes, the rinse water was drained. A total of three rinses were performed before the bags were removed. Excess water was gently pressed from the bags before being submerged in acetone. After 5 minutes, the acetone was gently pressed from the bags before overnight drying at RT. The next day, bags were dried at 110 °C for 4 hours before cooling in special desiccator pouches. The bags were weighed and % ADF was calculated as:

$$\frac{\text{Final bag weight} - (\text{Initial bag weight} \times \text{Blank bag correction})}{\text{Sample weight}} \times 100$$

Where,

Blank bag correction = (Final Blank bag weight/Initial Blank bag weight)

3.2.5.7 Nitrogen and carbon contents

Approximately 100 mg of previously ground samples were weighed in duplicate into foil cups. The cups were folded closed around the sample, forming a tear drop-shape. The samples were then loaded into the automatic sample carousel. Nitrogen and carbon contents of the samples were determined by complete combustion. NO_x and CO_2 gas contents were measured by infrared radiation in separate cells of the analyzer (LECO Corporation, St. Joseph, Michigan). The percent nitrogen and carbon was calculated from the previously entered sample weights by the computer.

3.2.5.8 Ash content

Approximately 2 g of ground sample was weighed in duplicate into 50 ml Pyrex beakers. The sample was ashed overnight at 500 °C before cooling to RT in a dessicator. The percent ash was calculated as:

$$\frac{\text{Total weight after ashing} - \text{Beaker weight}}{\text{Sample weight}} \times 100$$

3.2.6 Calculations

3.2.6.1 Digestibility

The digestibility of individual dietary components was determined using Celite® as an indigestible marker and analysis for acid insoluble ash (AIA) (McCarthy et al, 1977). The following formula using the AIA contents of feed (AIA_{feed}) and feces (AIA_{feces}) was used to calculate the nutrient digestibility of individual nutrients:

$$\text{Nutrient digestibility (\%)} = 1 - ((AIA_{feed} * Nutrient_{feces})/(AIA_{feces} * Nutrient_{feed}))$$

3.2.6.2 Volumes of gases

Litres of gases consumed and produced were calculated using the difference between recorded gas exchange values during the experiment and the room air values recorded immediately before and after the experimental period. The values recorded for each minute were corrected for drift of the analyzers, if statistically significant. The difference between the experimental and room air percent gas compositions was multiplied by the air flow out of the respiration chambers for each 30 minute period. Therefore, litres of gas consumed or produced were calculated for each 30 minute period, as follows:

V_{O2} (L/30 min) = total air flow (L)/30 min * (% O_2 room air - % O_2 test period)

V_{CO2} (L/30 min) = total air flow (L)/30 min * (% CO_2 test period - % CO_2 room air)

V_{CH4} (L/30 min) = total air flow (L)/30 min * (% CH_4 test period - % CH_4 room air)

3.2.6.3 Heat production

The formula by Brouwer (1965) was used to calculate heat production (HP) from indirect calorimetry. The formula was abbreviated by omitting the urinary nitrogen term because we planned for and attempted to collect urine, however the results were unsatisfactory and thus not included[7]. Therefore, the formula used to calculate HP from gas exchange was:

$$HP = (16.175 \times V_{O2}) + (5.02 \times V_{CO2}) - (2.17 \times V_{CH4})$$

[7] According to Weir (1949), the effect of ignoring the urinary nitrogen (i.e. protein metabolism) is 1% for every 12.3% of the total energy that was derived from protein.

where V_{O2}, V_{CO2}, and V_{CH4} represent volumes (L) of O_2 consumed and CO_2 and CH_4 produced, respectively. Daily HP was calculated as the summation of HP for each 30 minute of the 24 h period.

3.2.6.4 Respiratory quotient

The respiratory quotient (RQ) was calculated as:

$$RQ = \frac{V_{CO2} \text{ produced (L/30 minute period)}}{V_{O2} \text{ consumed (L/ 30 minute period)}}$$

3.2.6.5 Maintenance energy requirement

The maintenance energy requirement (ME_m) was calculated according to the formulae of Lodge et al (1979), for sows in positive energy balance:

$$ME_m = \frac{\text{total ME intake} - 1.43^1 \times \text{total ER}}{(\text{average weight})^{0.75}}$$

and, for sows in negative energy balance:

$$ME_m = \frac{\text{total ME intake} - 0.80^2 \times \text{total ER}}{(\text{average weight})^{0.75}}$$

where, total energy retention (ER) = total ME intake – HP

[1]: the value 1.43 is the reciprocal of the efficiency for utilization of ME from dietary sources (70%) reported by Verstegen et al (1971).

[2]: the value 0.80 is used because energy from body tissue replaces ME with an efficiency of 80% (Verstegen et al, 1971).

3.2.7 Statistical analysis

Data are presented as means ± SEM, unless otherwise stated. Values were considered significant at $P < 0.05$. Statistical analysis was performed using mixed procedure in SAS (SAS Inst. Inc., Cary, NC). The classification variable was feeding level and individual animals were treated as random variables. Model statements were tested using the Kenward-Roger degrees of freedom method. Least square means were compared using the 'pdiff' option.

3.3 Results

3.3.1 Sow body weight, daily gain, and feed intake

Sows fed 473 ± 5.5 kJ ME/$BW^{0.75}$ began the experiment weighing 175.7 ± 3.3 kg BW and ended the experiment at a final weight of 174.1 ± 3.2 kg, however the difference (-1.6 kg) was not significant (P=0.11). Sows fed 925 ± 6.1 kJ/$BW^{0.75}$ started at 174.1 ± 3.1 kg BW and gained ($P<0.0001$) weight up to 186.6 ± 3.3 kg[8] at the rate of 1292 ± 215 g/d. Actual daily feed intake was 1.84 ± 0.03 kg and 3.69 ± 0.05 kg for sows offered 473 kJ/$BW^{0.75}$ and 925 kJ/$BW^{0.75}$, respectively.

3.3.2 Nutrient digestibility

The digestibility of dietary energy and nutrients by the sows was determined by analysis of feed and feces and calculated according to the relative AIA contents (Table 3.3 and 3.4).

[8] Two sows were fed 925 kJ/$BW^{0.75}$ for a longer period (15 days) to avoid confounding effects of estrus on energy metabolism.

Table 3.3 Effect of feeding level (473 vs. 925 kJ/BW$^{0.75}$) on nutrient digestibility[1] by sows

	473 kJ/BW$^{0.75}$	925 kJ/BW$^{0.75}$		
Nutrient	**Digestibility[1]**	**Digestibility[1]**	**SEM**	**P-value**
Energy	79.7	80.9	0.8	0.34
Nitrogen	81.3	83.6	1.7	0.08
Carbon	81.6	82.3	1.2	0.74
Fat	87.3	85.6	3.0	0.73
NDF	42.3	61.2	5.9	0.11
ADF	24.4	50.6	4.9	0.03
Organic matter	84.1	86.1	0.9	0.09

[1]: least-square means

Table 3.4 Pooled means for nutrient digestibility of non-pregnant sows

Nutrient	Pooled mean	SEM
Energy	80.2	0.5
Nitrogen	81.2	1.1
Carbon	81.8	0.6
Fat	86.7	1.6
NDF	46.1	4.3
Organic matter	84.3	0.6

3.3.3 24 h heat production

Sows fed 473 kJ/BW$^{0.75}$ had an average daily heat production of 25.1 ± 0.4 MJ or 525 ± 9 kJ/BW$^{0.75}$. Sows fed 925 kJ/BW$^{0.75}$ had an average daily heat production of 31.2 ± 0.5 MJ or 624 ± 10 kJ/BW$^{0.75}$, which was significantly greater ($P<0.001$) than sows fed at 473 kJ/BW$^{0.75}$. HP was similar among sows receiving the same feeding level ($P=0.15$).

3.3.4 Calculated maintenance energy requirement

Based on the daily energy intakes and expenditures and using the formulae from Lodge et al (1979), ME_m was calculated for each individual animal (Table 3.5). For sows offered 473 kJ/BW$^{0.75}$, the mean calculated ME_m was 515 ± 8 kJ/BW$^{0.75}$ and, for sows offered 925 kJ/BW$^{0.75}$, the mean calculated ME_m was 495 ± 9 kJ/BW$^{0.75}$. The

calculated ME_m values were not significantly different (P=0.15). Therefore the overall mean of 506 ± 7 kJ/$BW^{0.75}$ was taken as the ME_m for this sample of the population.

Table 3.5 Energy intake, heat production, and calculated ME_m for individual sows

Sow	Energy intake (MJ/d)	Heat Production (MJ/d)	ME_m[1]
21:02	23.6	25.6	512
22:01	23.6	24.5	497
22:05	23.4	25.3	514
23:01	20.8	25.7	543
37:03	22.1	24.6	507
21:02	46.8	30.3	446[2]
22:01	46.8	32.7	520
22:05	46.8	31.1	477
23:01	44.5	29.5	480
37:03	47.1	31.7	501

[1]: ME_m calculated according to Lodge et al, 1979. [2]: This value was determined to be an outlier, according to Cook's distance and, therefore, was not included in the mean for 2x ME_m (495 ± 9 kJ/$BW^{0.75}$) or the overall mean (506 ± 7 kJ/$BW^{0.75}$).

3.3.5 Respiratory quotient

The mean daily respiratory quotient (RQ) for sows fed 473 kJ/$BW^{0.75}$ was 1.03 and was significantly lower (P<0.0001) than for sows fed 925 kJ/$BW^{0.75}$, where the mean daily RQ was 1.16 (SEM 0.02). The RQ of meal eating sows was 1.08 and 1.21 (SEM 0.08) for sows fed 473 and 925 kJ/$BW^{0.75}$, respectively, but was not different (P=0.28) by comparison. Two hours after ingesting the meal, sows fed 473 kJ/$BW^{0.75}$ had a RQ of 1.15 and sows fed 925 kJ/$BW^{0.75}$ had a RQ of 1.25 (SEM 0.04). The difference between the feeding levels was highly significant (P<0.0001). During fasting, the RQs decreased to 0.91[9] and 1.09 (SEM 0.02) and were different (P<0.0001) by comparison for sows fed 473 and 925 kJ/$BW^{0.75}$.

[9] The RQ for fasting is not available for 3 sows due to ventilation control failure which allowed the temperature of the room to drop below the normal range. A reduction in oxygen sensor measurements was detected in the final analysis of the data, but the reduction in oxygen measurements cannot be estimated.

Table 3.6 RQ of sows offered (473 kJ/BW$^{0.75}$ or 925 kJ/BW$^{0.75}$) during various prandial states (fasting, post-prandial, or meal eating) on the RQ of sows

Feeding level	Fasting[a]	2 h after meal	Meal eating	SEM	P-value
473 kJ/BW$^{0.75}$	0.91[a]	1.15[b]	1.08[a,b]	0.07	<0.0001
925 kJ/BW$^{0.75}$	1.09[a]	1.25[b]	1.21[b]	0.06	<0.05
SEM	0.02	0.04	0.08		
P-value	<0.001	<0.001	0.28		

[a,b]: values with different superscripts across rows represent differences P<0.05; Differences between feeding level were highly significant, except for meal eating which was not different (P=0.28).

3.4 Discussion

The pooled mean of the digestibility coefficient (DC) of energy for the sows in this experiment was 80.2 ± 0.5 % (Table 3.4). This compares to 80.2 % calculated from Noblet and Henry (1993) and the calculated values of 80.1 % and 81.1 % obtained from the two prediction equations in NRC (1998) based on the composition of the diet. However, the nutrient digestibility values determined from this experiment were different from values reported previously by Noblet and Shi (1993), who reported digestibility coefficients (DC) for sows for energy of 84.7 %, for organic matter of 86.6 %, for crude protein of 85.2 %, for NDF of 70.9 %, and for ADF of 60.4 %. Corresponding values from this experiment were: 80.2 ± 0.5 % for gross energy, 84.3 ± 0.6 % for organic matter, 81.2 ± 1.1 % for crude protein, 46.1 ± 4.3 % for NDF, and 30.4 ± 4.9 % for ADF (Table 3.4). The DC of ether extract (86.7 ± 1.6) was greater (P<0.0001) from this experiment than 69.1 % reported by Noblet and Shi (1993). The differences in DC from Noblet and Shi (1993) compared to current results may be due to differences among Canadian and European wheat and barley cultivars. Therefore, a comparison to Fairbairn et al. (1999) may be more appropriate given the significance of the barley fraction of the diet. Fairbairn et al. (1999) reported DC for barley of 4.4%,

52.6%, 62.8%, and 69.2% for ADF, NDF, EE, and CP, respectively. Therefore the digestibility of barley itself is lower than the digestibility of the mixed ration and may explain the lower digestibility observed in this experiment compared to the values reported by Noblet and Shi (1993).

The GE content of the diet was different ($P<0.05$) for sows fed 473 $kJ/BW^{0.75}$ (15.97 ± 0.04 MJ/kg) and 925 $kJ/BW^{0.75}$ (16.20 ± 0.10 MJ/kg), respectively. Sufficient diet for the entire experiment could not be stored as a single batch and, therefore, was prepared in two batches. A difference in the composition of the diet can be partially be explained by differences in carbohydrate content of the batches. The carbohydrate fraction of the diet is determined indirectly in proximate analysis. All other fractions are determined directly by laboratory analysis and summed. The difference between 100% and the laboratory analysis summation represents the carbohydrate fraction. There was a greater ($P<0.01$) ether extract fraction collected from the batch of diet for sows fed 473 $kJ/BW^{0.75}$ (2.47 ± 0.03) compared to the batch of diet for the sows fed 925 $kJ/BW^{0.75}$ (2.09 ± 0.05). There were with no other differences in laboratory analysis. This would result in a greater carbohydrate fraction by the difference in the batch of diet for the sows fed 925 $kJ/BW^{0.75}$.

The calculated ME_m values from this experiment were 515 ± 8 $kJ/BW^{0.75}$ and 495 ± 9 $kJ/BW^{0.75}$ for sows fed at 473 and 925 $kJ/BW^{0.75}$, respectively. These values were calculated using the formulae of Lodge et al (1979) and were comparable to previous reports (Close and Stanier, 1984). The calculated ME_m value for sows fed at approximately ME_m intake was numerically greater (by 20 kJ) than for sows fed at approximately twice ME_m intake. This phenomenon was previously demonstrated by

Wenk et al (1980) who reported a lower ME_m for ad libitum fed animals than restrictively fed animals and concluded that this was due to reduced physical activity. Animals fed ad libitum intake tend to exhibit reduced energy expenditure related to food foraging and similar activities. Halter et al (1980) reported the energy requirement for activity to be 30% higher for restrictively fed piglets compared to ad libitum fed piglets. The result of reduced physical activity is reduced HP and therefore the calculated ME_m is reduced. The calculated ME_m values do not represent *ad libitum* feed intake because the feed intake of the sows was restricted to below *ad libitum* intake, even at 925 $kJ/BW^{0.75}$.

Both of the ME_m values calculated from this experiment were greater than the values recommended by ARC (1981) of 458 $kJ/BW^{0.75}$ and by NRC (1998) of 106 $kcal/BW^{0.75}$ (equivalent to 444 $kJ/BW^{0.75}$). The fact that these values were greater than the current recommendations was expected, based on changes in body composition of modern sows (Merks, 2000; Ball et al, 2008). This was confirmed by the observed negative weight gain (-198 ± 96 g/d) of the sows fed at approximately ME_m (473 $kJ/BW^{0.75}$). Sows fed 473 $kJ/BW^{0.75}$ were in negative energy balance of -1980 kJ/d. Therefore, a new value for ME_m for sows of 506 ± 7 $kJ/BW^{0.75}$ is recommended to replace previously reported ME_m values.

If the entire weight loss of the sows is assumed to be due to loss of body fat, the energy lost would amount to 7.9 MJ/d. This was calculated based on the energy content of 39.7 kJ/g of body fat reported by Close and Stanier (1984). An apparent efficiency of energy utilization from body fat reserves of 0.25 was then calculated based on 7.9 MJ/d

body fat mobilization and 1.98 MJ/d additional energy required (i.e. 7.9 MJ/d mobilization ÷ 1.98 MJ/d required = 0.25).

Alternatively, the positive weight gain of sows fed for 925 kJ ME/$BW^{0.75}$ indicated positive energy balance for the sows of 15.3 ± 0.5 MJ/d. Again, if we assume that the entire weight gain was fat, the energy value of 1292 g/d of fat would be 51.3 MJ/d. The energy for fat gain (51.3 MJ/d) divided by the efficiency for adipose tissue accretion (0.76) reported by Campbell and Dunkin (1983) requires 67.5 MJ/d of energy intake above maintenance. However, because the calculated energy required for the entire 1292 g/d of weight gain as fat is greater than the excess energy from the energy balance calculation, this shows that the weight gain was both fat and protein.

In this experiment, the sows fed 925 kJ ME/$BW^{0.75}$ intake consumed 3.69 ± 0.05 kg of feed per day with a lysine content of 0.65%. The product of these values is 23.97 ± 0.35 g of lysine per day intake. The maintenance requirement for lysine is estimated at 36 mg/$BW^{0.75}$ (NRC, 1998) therefore the amount of lysine available for protein accretion above the maintenance requirement was 22.2 ± 0.3 g/d. Given that body protein is 7.08 % lysine (Möhn et al, 2000), this lysine intake would represent accretion of 313 ± 5 g/d of protein. Based on an energy cost of protein accretion of 44.4 kJ ME/g (NRC, 1998), 13.9 MJ ME would be utilized for a protein accretion of 313 g/d. The total daily ME intake was 46.07 MJ subtracting ME_m of 23.1 MJ ME leaves 23.0 MJ available for growth. Subtracting the energy cost of protein accretion of 13.9 MJ from the energy available for growth (23.0) leaves 9.1 MJ ME per day available for fat accretion. Because the energy cost of fat accretion is 52.3 kJ/g of fat, this excess energy (9.1 MJ) would result in 173 ± 4 g/d of fat accretion. In summary, the composition of

gain calculated in this manner would be 313 g/d protein and 173 g/d fat for a total of 485 ± 8 g/d. Protein is 75% water, therefore 313 g/d of protein would total to 1252 g/d of body protein. Likewise, fat is 10% water, therefore 173 g/d of adipose tissue would total to 193 g/d. Thus the total body weight gain would be 1252 g/d of protein plus 193 g/d adipose tissue for total gain of 1444 g/d. This predicted gain is numerically greater than the measured average daily gain of 1292 ± 215 g/d. Therefore some of these values, as derived from the literature, must be incorrect. The most likely values are those for ME_m and lysine maintenance requirement due to the changes in body composition of modern sows in comparison to the animals used to determine the current recommendations. Sow body weight and lean tissue mass have increased and both raise maintenance energy and protein requirements due to the process of protein turnover (Ball et al, 2008).

ME_m is greater than previous estimates (ARC, 1981; NRC, 1998) due to the changes in body composition of modern sows. Modern breeds have greater lean tissue percentage in their carcasses. Lean tissue (i.e. protein) is constantly synthesized and catabolized in the process of protein turnover. This process requires energy and amino acids, contributing to the energy and amino acid requirements. Wenk et al (1980) previously reported that ME_m values have increased for swine due to the increase in lean tissue turnover.

The null hypothesis of this experiment was that "HP will not be different between sows fed for 2.0 times ME_m and sows fed for 1.0 times ME_m". The measured HP of sows fed for twice ME_m intake was 31.1 ± 0.5 MJ/d and was greater ($P<0.0001$)

than for sows fed for ME_m intake at 25.1 ± 0.4 MJ/d. Therefore, this hypothesis was rejected.

3.5 Conclusions and implications

The results of this experiment indicate that current estimates of sow ME_m are too low. This was shown for sows fed at approximately ME_m and for sows fed at approximately twice ME_m. Due to changes in body composition, it has also been suggested that protein maintenance requirements are too low (Wenk et al, 1980). Therefore, sow maintenance energy and protein requirements should be increased to reflect the requirements of modern sows. A new ME_m was calculated to be 506 kJ $ME/BW^{0.75}$ for this population of sows.

3.6 Literature cited

Agricultural Research Council. 1981. The nutrient requirements of pigs. 1st ed. Slough, U.K, Commonwealth.

Ball, R. O., R. S. Samuel, & S. Moehn. 2008. Nutrient requirements of prolific sows. Advances in Pork Production. 19: 223-236.

Brouwer, E. 1965. Report of Sub-Committee on Constants and Factors. Pages 441-443 in Proceedings of the 3rd Symposium on Energy Metabolism. Blaxter, K. L., ed. 11th ed. EAAP Publication, Academic Press, New York, NY.

Brown-Brandl, T. M., J. A. Nienaber, H. Xin, & R. S. Gates. 2004. A literature review of swine heat production. T. ASAE. 47(1): 259-270.

Campbell, R. G., & A. C. Dunkin. 1983. The effects of energy intake and dietary protein on nitrogen retention, growth performance, body composition and some aspects of energy metabolism of baby pigs. Br. J. Nutr. 49: 221-230.

Close, W. H., & M. W. Stanier. 1984. Effects of plane of nutrition and environmental temperature on the growth and development of the early-weaned piglet. 2. energy metabolism. Anim. Prod. 38: 221-231.

Fairbairn, S. L., J. F. Patience, H. L. Classen, & R. T. Ziljstra. 1999. The energy content of barley fed to growing pigs: Characterizing the nature of its variability and developing prediction equations for its estimation. J. Anim. Sci. 77: 1502-1522.

Foxcroft, G. R. 2008. Hyper-prolificacy and acceptable post-natal development - a possible contradiction. Advances in Pork Production. 19: 205-211.

Halter, H. M., C. Wenk, & A. Schürch. 1980. Effect of Feeding Level and Feed Composition on Energy Utilization, Physical Activity and Growth Performance of Piglets. Pages 395-398 in Energy Metabolism. Mount, L. E., ed. EAAP Publ. No. 26 ed. Butterworths, London.

Kempster, T., A. Cuthbertson, & G. Harrington. 1982. Carcase evaluation in livestock breeding, production and marketing. . Great Britain, Granada Publishing Ltd.

Lodge, G. A., D. W. Friend, & M. S. Wolynetz. 1979. Effect of pregnancy on body composition and energy balance of the gilt. Can. J. Anim. Sci. 59: 51-61.

Merks, J. W. M. 2000. One Century of Genetic Changes in Pigs and the Future Needs. Pages 8-19 in The Challenge of Genetic Change in Animal Production. Hill, W. G., S. C. Bishop, B. McGuirk, J. C. McKay, G. Simon & A. J. Webb, eds. 27th ed. Occ. Publi. Br. Soc. Anim. Sci., BSAS, Edinburgh, UK.

Möhn, S., A. M. Gillis, P. J. Moughan, & C. F. M. de Lange. 2000. Influence of dietary lysine and energy intakes on body protein deposition and lysine utilization in the growing pig. J. Anim. Sci. 78: 1510-1519.

National Research Council. 1998. Nutrient requirements of swine. 10th ed. Washington, D.C., National Academy Press.

Noblet, J., & W. H. Close. 1980. The Energy Costs of Pregnancy in the Sow. Pages 335-339 in Energy Metabolism. Mount, L. E., ed. EAAP. Publ. 26. ed. Butterworths, London.

Noblet, J., & Y. Henry. 1993. Energy evaluation systems for pig diets: A review. Livest. Prod. Sci. 36: 121-141.

Noblet, J., & X. S. Shi. 1993. Comparative digestibility of energy and nutrients in growing pigs fed ad libitum and adult sows fed at maintenance. Livest. Prod. Sci. 34: 137-152.

Verstegen, M. W. A., A. J. H. van Es, & H. J. Nijkamp. 1971. Some aspects of energy metabolism of the sow during pregnancy. Anim. Prod. 13: 677-683.

Wenk, C., H. P. Pfirter, & H. Bickel. 1980. Energetic aspects of feed conversion in growing pigs. Livest. Prod. Sci. 7: 483-495.

4.0 THE HEAT INCREMENT OF FEEDING WITH RESPECT TO FEEDING FREQUENCY

4.1 Introduction

Postprandial thermogenesis (Diamond et al, 1985), thermic effect of feeding (TEF) (D'Alessio et al, 1988; Bellisle et al, 1997), and heat increment of feeding (HIF) (Noblet and Henry, 1993; Rosen and Trites, 1997) are all common terms for the same biological phenomenon; the quantity of heat released due to the energy requirement of digestive and metabolic processes associated with ingestion and digestion of a meal. The HIF has been defined in sows as the measured component of the heat production (HP) of an individual sow above basal metabolism (Rosen and Trites, 1997). However, it can be difficult to determine an exact value for the HIF in swine, especially due to the physical activity associated with eating in these animals. Accurate determination of the HIF is important because it is used to calculate the net energy (NE) of a particular diet or feedstuff (i.e. ME – HIF = NE) (Noblet et al, 1985). As a result of the difficulties associated with the measurement of the HIF in swine, NRC (1998) concluded that "the ratio of NE to ME ranged from 0.66 to 0.75".

The HIF is a result of eating and digesting, therefore the number of meals, or feeding frequency, must be considered in the determination of HIF. Feeding frequency has been studied quite extensively in the laboratory rat using indirect calorimetry, which also provides information about substrate utilization (respiratory quotient (RQ)). Laboratory rats are normally "nibblers", meaning they prefer to consume numerous small meals and eat almost continuously. When access to food is limited, rats, and other

nibbling species, such as pigs, can be trained to consume their daily ration over short periods of time. Depending on the infrequency of feeding, the consumption pattern may be classified into "meal feeding", "intermittent feeding", or "gorging" classes (Fabry and Tepperman, 1970). Sows are not continuously fed, therefore the effect of feeding pattern on HP must be measured to accurately determine ME_m.

Bellisle et al (1997) and Moehn et al (2004) reported that feeding frequency did not have an effect on HIF humans and in pigs, respectively. Greater energy expenditure occurred at the time of feeding than during the fasting or postprandial states in sows, regardless of size or frequency of meals. LeBlanc and Diamond (1986) reported that even the sight and/or smell of a meal led to equivalent metabolic HIF responses in dogs. Greater HP was measured during the postprandial state in frequently fed sows than for the meal fed sows in the experiment by Moehn et al (2004) indicating continued HIF effects due to frequent feeding. Physical activity was likely reduced as a result of meal feeding and thus led to reduced energy expenditure (Fabry and Tepperman, 1970). Ramonet et al (2000) reported reduced physical activity of sows fed fibrous diets, thus reducing HP. Many authors (Leveille and Hanson, 1965; Baird, 1970; Allee et al, 1972; Han, 1973; Ozelci et al, 1977) have reported that meal-fed animals are more energy efficient than nibbling animals.

Modern pork production systems typically feed sows once per day, as suggested in the "Recommended code of practice for the care and handling of farm animals: Pigs" (1993). However, the advent of computerized feeding systems and otherwise highly mechanized feeding of sows will facilitate different feeding frequencies without increased labour. Therefore, it is relevant to investigate the energy requirements of sows

under different feeding frequencies to determine the feeding pattern that optimizes feed efficiency. Equally important, frequent feeding is proposed to be used for future studies of nutrient requirements using the indicator amino acid oxidation method with oral delivery of isotope (Moehn et al, 2004). Therefore it is essential that the impact of increased feeding frequency be quantified so that, if necessary, correction factors may be applied.

The primary objective of this study was to compare the HIF, and thus the efficiency of energy retention, of nibbling versus meal fed sows. The daily HP of the sows was determined by indirect calorimetry. The 24 h values were extrapolated from the mean HP (kJ/min) of individual 'states'. Using the HIF and the measured DE content of the diet, the NE content of the diet was also determined. Sows received feed at two levels, approximately ME_m and twice the ME_m energy intake and at two feeding frequencies (nibbling and meal fed). Therefore the null hypothesis "the heat increment of feeding, and therefore the NE content of the diet, will not be different between sows being fed frequent, small meals compared to sows fed large meals" will be tested.

4.2 Materials and Methods

The animals, ethics approval, diets, feeding, housing, and chemical analyses were the same as used in Chapter 3. See Chapter 3.2 for details.

4.2.1 Statistical analysis

Data are presented as means ± SEM, unless otherwise stated. Values were considered significant at $P < 0.05$. Statistical analysis was performed using the mixed procedure in SAS 9.1 (SAS Inst. Inc., Cary, NC). The classification variables were. feeding level and physiological 'states' (fasting, nibbling, meal-fed, and postprandial). Model statements were tested using the Kenward-Roger degrees of freedom method. Individual animals were treated as random variables. Least square means were compared using the 'pdiff' option.

4.3 Results

4.3.1 Feeding frequency and feeding level effect on heat production

Sows eating small meals (nibbling) had greater ($P<0.01$) HP than sows fasting or after the large meal (postprandial). Meal eating sows had similar heat production, regardless of feeding level ($P=0.55$). The mean 24 h HP of sows at both feeding levels by feeding frequency are shown in Table 4.1. Overall, when the sows were nibbling, their HP was greater ($P<0.0001$) than during the fasting or postprandial 'states' and not different ($P>0.1$) than when eating a single meal.

Table 4.1 Mean 24 h heat production of sows by feeding level and prandial state

Feeding level	Fasting (kJ/d)	Nibbling (kJ/d)	Postprandial (kJ/d)	Meal eating (kJ/d)	SEM	P-value
473 kJ ME/$BW^{0.75}$	23.9 [b]	30.4 [c]	20.9 [a]	27.9 [b,c]	2.6	<0.01
925 kJ ME/$BW^{0.75}$	29.1 [a]	35.6 [c]	29.4 [a]	31.5 [b,c]	2.6	<0.0001
SEM	1.1	0.8	0.7	4.1		
P-value	<0.0001	<0.0001	<0.0001	0.55		

[a,b,c]: Values with the different letters across a row represent differences within feeding level of P<0.01. Comparisons between feeding level were significantly different, except for meal eating (P=0.55)

4.3.2 Feeding frequency and feeding level effect on respiratory quotient

Nibbling and meal fed sows had lower (P<0.05) RQs than fasting or postprandial sows for both feeding levels. There was trend towards a greater RQ in sows fed twice ME_m as compared to sows fed at ME_m (Table 4.2)

Table 4.2 Respiratory quotient of sows by feeding level and feeding frequency

Feeding level	Fasting	Nibbling	Postprandial	Meal eating	SEM	P-value
473 kJ ME/$BW^{0.75}$	0.91 [a]	0.96 [a]	1.15 [b]	1.08 [a,b]	0.07	<0.01
925 kJ ME/$BW^{0.75}$	1.09 [a]	1.12 [a,c]	1.25 [b]	1.21 [b,c]	0.07	<0.05
SEM	0.02	0.03	0.04	0.08		
P-value	<0.05	<0.0001	<0.0001	0.28		

[a,b,c]: Values with the different letters across a row represent differences within feeding level of P<0.05. Feeding level had a significant effect on RQ within each feeding frequency, except for meal eating, which was not different (P=0.28).

4.4 Discussion

These sows exhibited greater RQs than might be expected; RQ should be lower than one due to body lipid mobilization and oxidation for energy. However, Tepperman et al (1943) reported a significant amount of time was required for the adaptation of rats to new feeding frequencies. As a result, the animals continued to exhibit characteristics of meal fed animals with respect to RQ even when the feeding frequency had been changed. Specifically, two groups of rats were sedated and a bolus of glucose given. In

both sets of rats, the RQ was greater than one, as expected due to the bolus feeding of a significant amount of glucose. However, in the rats adapted to meal feeding, the RQ was significantly greater than for the rats not adapted to meal feeding suggesting more efficient storage of excess energy by lipogenesis. In the rats previously adapted to the nibbling feeding frequency, the RQ was only slightly greater than one, indicating that oxidation was the primary disposal mechanism for the excess glucose. Although the effect was significant in a first experiment, Tepperman et al (1943) accentuated the effect by reducing the feeding frequency of the rats from a single meal over three hours per day down to a single meal in one hour. The result was much greater RQ values for the rats adapted to meal feeding than for rats not adapted to meal feeding due to the bolus glucose. Because of the significant adaptation time required to adjust to new feeding frequencies, the sows in this study would not have been adapted to the frequent feeding frequency. Sows were fed once per day until started in the study and then were fed twice per day, except on study days. Therefore sows were adapting to being fed twice per day versus once per day and were not adapted to the nibbling feeding frequency that was imposed only on study days. Given that the sows' metabolism was well adapted to once per day feed intake and efficient storage of the energy, it is not surprising, even though energy intake was lower than ME_m, that sows fed 473 $kJ/BW^{0.75}$ exhibited RQs greater than and equal to one while eating and digesting each meal. Allee et al (1972) demonstrated the hyperlipogenic capacity of pig adipose tissue due to meal eating. This lends further support to the conclusion that significant lipogenesis occurred in the sows during feeding, regardless of the feeding frequency.

RQ values greater than one during fasting do not, however, have a biological explanation. Measured RQ for the animals fed 473 kJ/$BW^{0.75}$ averaged 1.02 during fasting for five animals and 0.91 for two animals. Due to a ventilation control malfunction that allowed the temperature to drop during the overnight fast for three sows, the oxygen values during this period are lower than expected. Therefore, we could expect that the RQ value actually dropped below one during the overnight fast, as expected, but we were not able to truly measure this effect in three of the sows. In future studies, we will include a temperature correction measurement parameter to adjust for changes in room air temperature.

The 24 h HP of sows was affected by the different feeding frequencies in a similar manner for both of the feeding levels. When sows were consuming small meals every 30 minutes, HP was greater ($P<0.01$) than when sows were fasting or following the large meal (postprandial). The HP of the eating sows, whether consuming the frequent, small meals or consuming the single large meal was not different. A portion of the greater HP associated with eating is due to the physical activity of sows when consuming a meal. Sows tend to be quite enthusiastic about feeding and will consume their meals rapidly while standing. The other portion of the greater HP associated with eating is due to the heat increment of feeding (HIF).

The difference between the ME content of a feedstuff or complete ration and the net energy (NE) content of the same feedstuff or ration is the HIF. Therefore, accurate measurement of the NE content of feedstuffs can be achieved by accurately determining the HIF associated with digestion of particular feedstuffs. However, the accurate measurement of HIF is somewhat elusive, leading to a large range of values. Further,

NE values of feeds can only be accurately applied under the same conditions under which they were determined (de Lange and Birkett, 2005). van Milgen et al. (2001) reported that the efficiency of NE for protein synthesis and catabolism are equivalent in growing pigs. The difference between the HP and net energy for maintenance (NE_m) is the HIF when the ration is fed at maintenance energy intake.

Noblet and Henry (1993) reported that the calculation of NE:ME ratios is dependent on the situation. Sows in this study were fed 473 kJ/$BW^{0.75}$ and lost weight (-198 g/d) and were, therefore, not being fed at ME_m which resulted in negative energy balance. Sows fed 925 kJ/$BW^{0.75}$ were in positive energy balance therefore a portion of the additional ME intake was retained as protein and fat.

The NE content of the diet, calculated according to Equation 3 Table 6 of Noblet et al (1994), was 8.72 ± 0.06 MJ/kg for sows fed 473 kJ/$BW^{0.75}$ and 9.06 ± 0.10 MJ/kg for sows fed 925 kJ/$BW^{0.75}$. The NE content of the feed was greater ($P<0.01$) for sows fed 925 kJ/$BW^{0.75}$ which was expected given the difference in the GE content of the feeds. Therefore total NE intakes were 16.1 ± 0.3 MJ NE/d and 33.4 ± 0.3 MJ NE/d for sows fed 473 kJ ME/$BW^{0.75}$ and 925 kJ ME/$BW^{0.75}$, respectively. The NE content of the feed calculated using NE=ME-(HIF) where HIF=HP-FHP (Noblet, 2006) and where FHP was estimated to be 16.6 ± 0.2 MJ/d (750 kJ/$BW^{0.60}$) from Noblet et al, 1994 is 7.5 ± 0.3 MJ/kg or 13.8 ± 0.7 MJ NE/d and 8.7 ± 0.1 MJ NE/kg or 32.4 MJ NE/d. The FHP was determined from sows fed 473 kJ/$BW^{0.75}$ during this experiment 8 h after the meal using the same 5 h period for three sows. Two of the sows had significant changes in HP during the period, so the data was not used. This period was chosen because no spikes in HP were noted, indicating that these three sows did not stand up during the

overnight period and, being that the sows were fed approximately ME_m intake, a sufficiently long enough fasting state was achieved. Therefore, the FHP of these three sows was determined to be 17.9 ± 0.9 MJ ME/d or 796 ± 41 $kJ/BW^{0.60}$. The value for FHP determined in this experiment was not different from 750 $kJ/BW^{0.60}$ previously reported by Noblet et al (1994). Using 17.9 ± 0.9 MJ ME/d as FHP in NE=ME-(HP-FHP) calculates an NE value for the feed of 8.4 ± 0.7 MJ/kg for a total intake of 15.7 ± 1.2 MJ NE/d for sows fed for ME_m. NE content of the feed was not different from the value predicted by Noblet et al (1994) equation 3 and that determined using the experimental FHP.

By simple subtraction, according to the equation HIF=HP-FHP, it was possible to determine if there was a difference in the HIF due to the feeding frequency of nibbling compared to meal eating. The extrapolated 24 h value for HP for ME_m sows during nibbling was 30.3 ± 0.8 MJ ME/d and during the meal eating it was 30.7 ± 6.4 MJ ME/d. These values are not different, therefore the HIF calculated from HIF=HP-FHP would not be different. In a similar manner, during nibbling, sows fed twice ME_m had 24 h HP of 35.6 MJ ME/d and during meal eating 31.5 MJ ME/d. Again, these values were not different, therefore calculated HIF would not be different. This result agrees with the review by Bellisle et al (1997) who concluded "that there is no strong evidence in support of a biologically-significant difference in [HIF] according to meal patterns". Bellisle et al (1997) also concluded that meal pattern had no effect on the total energy expenditure.

The null hypothesis of this experiment was that "the heat increment of feeding will not be different between sows being fed frequent, small meals compared to sows

fed large meals". The calculated HIF of sows while eating, whether frequently fed or meal fed was not different ($P>0.05$). Therefore, this hypothesis cannot be rejected.

4.5 Conclusions and Implications

The 24 h HP of sows extrapolated from the period of frequent feeding or meal eating was not different. This indicates that there was no difference in the HIF of sows when consuming equal amounts of feed in different feeding frequencies. The implication for industry is that whether sows are fed frequently (i.e. many small meals from electronic feed stations) or fed once per day, the energy of the meal is neither used more nor less efficiently due to the energy costs associated with ingestion and digestion of the meal.

Regardless of feeding frequency, energy metabolism is equivalent. Therefore, frequent meal feeding, as suggested by Moehn et al (2004) as the best conditions for indicator amino acid oxidation and energy metabolism studies due to the stability of carbon dioxide values, are equally valid with respect to daily HP and RQ measurements.

4.6 Literature Cited

Allee, G. L., D. R. Romsos, G. A. Leveille, & D. H. Baker. 1972. Metabolic adaptation induced by meal-eating in the pig. J. Nutr. 102: 1115-1122.

Baird, D. M. 1970. Individual and "skip-day" vs. group feeding systems for sows during gestation. Research Report 82 ed. College of Agriculture Experiment Stations, University of Georgia.

Bellisle, F., R. McDevitt, & A. M. Prentice. 1997. Meal frequency and energy balance. Br. J. Nutr. 77(Suppl. 1): S57-S70.

D'Alessio, D. A., E. C. Kavle, M. A. Mozzoli, K. J. Smalley, M. Polansky, Z. V. Kendrick et al. 1988. Thermic effect of food in lean and obese men. J. Clin. Invest. 81: 1781-1789.

de Lange, C. F. M., & S. H. Birkett. 2005. Characterization of useful energy content in swine and poultry feed ingredients. Can. J. Anim. Sci. 85: 269-280.

Diamond, P., L. Brondel, & J. LeBlanc. 1985. Palatability and postprandial thermogenesis in dogs. Am. J. Physiol. Endocrinol. Metab. 248: E75-E79.

Fábry, P., & J. Tepperman. 1970. Meal frequency - a possible factor in human pathology. Am. J. Clin. Nutr. 23(8): 1059-1068.

Han, I. K. 1973. Effect of frequency of meal on growth rate, nutrient digestibility, body composition, nitrogen retention and heat production of rats. Nutr. Rep. Int. 7(1): 9-18.

LeBlanc, J., & P. Diamond. 1986. Effect of meal size and frequency on postprandial thermogenesis in dogs. Am. J. Physiol. 250: E144-E147.

Leveille, G. A., & R. W. Hanson. 1965. Influence of periodicity of eating on adipose tissue metabolism in the rat. Can. J. Physiol. Pharmacol. 43(6): 857-868.

Moehn, S., R. F. P. Bertolo, P. B. Pencharz, & R. O. Ball. 2004. Pattern of carbon dioxide production and retention is similar in adult pigs when fen hourly, but not when fed a single meal. BMC Physiology. 4: 11-18.

National Research Council. 1998. Nutrient requirements of swine. 10th ed. Washington, D.C., National Academy Press.

Noblet, J. 2006. Recent Advances in Energy Evaluation of Feeds for Pigs. Pages 1-26 in Recent Advances in Animal Nutrition. Garnsworthy, P. C., & J. Wiseman, eds. Nottingham University Press, Nottingham.

Noblet, J., H. Fortune, X. S. Shi, & S. Dubois. 1994. Prediction of net energy value of feeds for growing pigs. J. Anim. Sci. 72: 344-354.

Noblet, J., & Y. Henry. 1993. Energy evaluation systems for pig diets: A review. Livest. Prod. Sci. 36: 121-141.

Noblet, J., J. Le Dividich, & T. Bikawa. 1985. Interaction between energy level in the diet and environmental temperature on the utilization of energy in growing pigs. J. Anim. Sci. 61(2): 452-459.

Ozelci, A., D. R. Romsos, & G. A. Leveille. 1977. Influence of diet composition on nitrogen balance and body composition in meal-eating and nibbling rats. J. Nutr. 107: 1768-1774.

Ramonet, Y., J. van Milgen, J. Y. Dourmad, S. Dubois, M. C. Meunier-Salaun, & J. Noblet. 2000. The effect of dietary fibre on energy utilisation and partitioning of heat production over pregnancy in sows. Br. J. Nutr. 84(1): 85-94.

Rosen, D. A. S., & A. W. Trites. 1997. Heat increment of feeding in stellar sea lions, *Eumetopias jubatus*. Comparative Biochemistry and Physiology Part A: Physiology. 118: 877-881.

Tepperman, J., J. R. Brobeck, & C. N. H. Long. 1943. The effects of hypothalamic hyperphagia and of alterations in feeding habits on the metabolism of the albino rat. Yale J. Biol. Med. 15: 855-874.

Wenk, C., P. C. Colombani, J. van Milgen, & A. Lemme. 2001. Glossary: Terminology in Animal and Human Energy Metabolism. Pages 409-421 103rd ed. EAAP Publication, Wageningen Pers.

5.0 THE 24 h HEAT PRODUCTION OF PREGNANT SOWS AT EARLY-, MID-, AND LATE-GESTATION

5.1 Introduction

Modern sows are more prolific than their predecessors in both the numbers of piglets born per litter and the size of the piglets produced. Recently the milestone of 30 piglets weaned per sow within one year was achieved through maximizing the number and size of litters produced each year (Jensen and Peet, 2006). Maximal litter size and growth rate, re-breeding success, and, ultimately the longevity of the sow in the production system, require that sows maintain adequate body condition through a series of gestation and lactation cycles (Noblet et al, 1990). The first step in providing adequate energy nutrition for modern, high producing sows is to determine the energy requirement during gestation (Walach-Janiak et al, 1986).

Energy requirements are divided into maintenance and growth by the factorial approach of NRC (1998). Energy requirements of pregnant sows are further divided to account for the energy required by the products of conception (including developing fetuses). In a similar manner, heat production (HP) can be apportioned into the two fractions: 1) from the sow and 2) from the products of conception. Dividing the total HP into these fractions allows the determination of the energy requirement of the sow versus the energy requirement of the products of conception (NRC, 1998). Verstegen et al (1987) stated that "the feed requirement for fetal development is small" compared to the energy required for maintenance and growth during gestation. Quantification of the

energy intake required for maternal and fetal development is necessary to ensure productivity and longevity of the sow.

HP measured by indirect calorimetry is calculated according to the formula of Brouwer (1965) based on measured volumes of oxygen consumed and carbon dioxide and methane produced. The respiratory quotient (RQ) is calculated as the volume of carbon dioxide (V_{CO2}) expired divided by the volume of oxygen (V_{O2}) consumed. According to Simonson and DeFronzo (1990) a RQ value of 0.705 is indicative of adipose tissue oxidation, a RQ value of 1 is indicative of carbohydrate oxidation. RQ values above 1 indicate active storage of energy by lipogenesis. The combination of measured HP and RQ is invaluable in the determination of energy metabolism.

Pregnancy is the most common condition of sows in modern pork production. Sows are bred for maximum reproductive capacity in both the number and size of the litters. Sows are capable of producing 2.5 litters per year based on 115 days of gestation and 21 days of lactation. Therefore, nearly 80% of the year sows are pregnant (Lucia et al, 1999). Sows are kept in production for an average of 4 litters in US breeding herds (Rodriguez-Zas et al, 2006) and 3.8 litters in top Canadian herds (Foxcroft et al, 2005). There are a number of reasons to increase sow longevity including: 1) the opportunity to recover the initial economic cost of breeding stock, 2) improved productivity (i.e. litter size and weight), and 3) improved disease resistance (Rodriguez-Zas et al, 2006). Therefore, feeding pregnant sows for optimal maternal and fetal development and longevity is essential for efficient pork production.

The objectives of this study were: 1) to measure the daily HP and average RQ of sows on Days 30, 45, and 105 of gestation by indirect calorimetry, 2) to calculate an

exponent correlating HP to BW, and 3) to partition HP between the sow and products of conception.

5.2 Materials and methods

The animals, ethics approval, diets, housing, and chemical analyses were the same as used in Chapter 3.0. However, the sows (n=5, 186.6 ± 3.5 kg) were bred and confirmed pregnant at the outset of this study. Respiration data collection was not performed on one animal for Day 105 due an injury. Therefore, data reported for Day 105 are only for four animals.

5.2.1 Feeding

Sows were fed one-half of their daily feed allowance twice daily. The diet formulation was the same as previously described (Table 3.1) and analyzed as previously described (Table 5.1). Daily feed intake was determined based on body weight and backfat measured post-breeding and the recommendations of Aherne et al (1999) for a desired amount of backfat gain.

Table 5.1 Proximate analysis of the diet as-fed during gestation (least-square means)

	Day 30		Day 45		Day 105			
Analyzed	**N**	**Mean**	**n**	**Mean**	**n**	**Mean**	**SEM**	**P-value**
GE, MJ/kg	3	16.11[a]	3	16.44[b]	2	16.41[b]	0.15	<0.05
CP, %	5	14.8[a]	5	15.8[b]	2	15.2[b]	0.3	<0.05
Fat, %	5	1.86[a]	5	2.23[b]	2	2.41[b]	0.07	<0.05
Carbon, %	5	38.3	5	38.8	2	37.8	0.4	0.14
NDF, %	5	14.3	5	14.8	1	14.8	0.7	0.47
ADF, %	5	5.57	5	5.31	2	5.77	0.23	0.27
AIA, %	3	1.39	5	1.44	2	1.37	0.08	0.71
Ash, %	5	6.72	5	6.71	2	6.72	0.01	0.99

5.2.2 Calculations

5.2.2.1 Digestibility

The digestibility of individual dietary components was determined using Celite® as an indigestible marker analyzed as acid insoluble ash (AIA) (McCarthy et al, 1977). The following formula using the AIA contents of feed and feces was used to calculate the percent digested of individual nutrients:

$$\% \text{ digested} = 1 - ((AIA_{feed} * Nutrient_{feces}) / (AIA_{feces} * Nutrient_{feed}))$$

5.2.2.2 Heat production (HP)

The formula of Brouwer (1965) was used to calculate HP from indirect calorimetry. The formula was abbreviated to the following because quantitative collection of urine required for the full formula was attempted, but the analysis was unsatisfactory. Therefore, the formula used to calculate HP from gas exchange was:

$$HP = 16.175 * O_2(l) + 5.02 * CO_2(l) - 2.17 * CH_4(l);$$

5.2.2.3 Respiratory quotient (RQ)

The respiratory quotient was calculated as:

$RQ = CO_2$ produced (L/day) / O_2 consumed (L/day)

5.2.2.4 Energy intake/BW^x

Total daily energy intake expressed per unit metabolic live weight was calculated:

Energy intake/$BW^{0.75}$ = (kg of feed/d x energy content of feed) / $BW^{0.75}$

Similarly, energy intake was corrected for BW using the exponent 0.6116 which was calculated from this experiment:

Energy intake/$BW^{0.6116}$ = (kg of feed/d * energy content of feed) / $BW^{0.6116}$

5.2.2.5 HP/BW^x

Total 24 h HP expressed per unit metabolic live weight was calculated:

$HP/BW^{0.75}$ = 24 h HP / $BW^{0.75}$

Similarly, 24 h HP was corrected for BW using the exponent 0.6116 which was calculated from this experiment:

$HP/BW^{0.6116}$ = 24 h HP / $BW^{0.6116}$

5.2.2.6 Calculated litter weight (kg)

The weight of the litter was calculated for respiration days according to the formula from McPherson et al (2004), modified to include number of live piglets born:

Litter weight = live piglets born * (0.00108*day of gestation^3 – 62.922)

5.2.2.7 Calculated weight of products of conception (kg)

The weight of the products of conception was calculated assuming 19.8 g/d gain per piglet (NRC, 1998):

Weight of products of conception = day of gestation * live piglets born * 19.8 g/d

5.2.2.8 Calculated maternal BW (kg)

The maternal BW of the sows was calculated for the respiration days:

Maternal BW = measured BW – (weight of products of conception)

5.2.2.9 Maternal ME_m HP (MJ/d)

The HP related to the ME_m for the maternal BW was predicted using the value 506 kJ/$BW^{0.75}$ previously determined (Chapter 3.0):

Maternal ME_m HP = maternal $BW^{0.75}$ * 0.506 MJ/$BW^{0.75}$

5.2.2.10 Total BW ME_m HP (MJ/d)

The HP related to the ME_m for the total measured BW was predicted using the value 506 kJ/BW0.75 previously determined (Chapter 3.0):

Total ME_m HP = measured $BW^{0.75}$ * 0.506 MJ/$BW^{0.75}$

5.2.3 Statistical analysis

Data are presented as means ± SEM, unless otherwise stated. Values were considered significant at $P < 0.05$. Statistical analysis was performed using the mixed procedure in SAS 9.1 (SAS Inst. Inc., Cary, NC). Model statements were tested using the Kenward-Roger degrees of freedom method. The classification variable 'day' was used and individual animals were treated as random variables. Differences between least square means were estimated using the 'pdiff' option. Non-linear regression was used to calculate an exponent that correlated HP and BW according to the model: HP = BW^x.

5.3 Results

5.3.1 Nutrient digestibility

The digestibility of energy and nutrients from the diet was determined by analysis of feed and feces for Celite® content. Pooled means for values not significantly different between the 'days' of gestation are listed in Table 5.2.

Table 5.2 Nutrient digestibility least square means and P-values for gravid sows on three 'days' of gestation

	Day 30[1]	Day 45[1]	Day 105[2]		
Nutrient	**Nutrient digestibility**	**Nutrient digestibility**	**Nutrient digestibility**	**SEM**	**P-value**
Energy	82.0	78.0	82.6	2.2	0.14
Nitrogen	80.5	73.2	82.6	0.04	0.29
Carbon	90.0	85.4	89.0	0.02	0.31
NDF	52.9	28.5	49.7	0.22	0.46
Organic Matter	85.7	79.0	85.5	4.0	0.26
Fat	91.3^{b}	$82.1^{a,c}$	$89.2^{b,c}$	2.1	<0.05

[1]: 5 sows; [2]: 4 sows; [a,b,c]: values with different superscripts across a row represent differences $P<0.05$.

Table 5.3 Pooled means of nutrient digestibility for gravid sows on three 'days' of gestation

Nutrient	Pooled mean	N	SEM
Energy	80.6	8	1.1
Nitrogen	77.7	9	2.3
Carbon	80.7	5	1.2
NDF	39.8	8	8.0
ADF	20.3	9	7.8
Organic matter	82.6	9	1.8

Table 5.4 Means for daily gain (g/d) of gravid sows between study 'days' of gestation and body weight, 24 h HP, daily energy intake, RQ, metabolic HP, and metabolic energy intakes of gravid sows on study 'days' of gestation

Parameter	Day 30 [1]	Day 45 [1]	Day 105 [2]	P-value
Total daily gain (g/d)	274 ± 28^{a}	389 ± 90^{a}	609 ± 34^{b}	<0.05
BW on respiration day (kg)	196.2 ± 3.6^{a}	202.4 ± 3.5^{b}	237.5 ± 5.1^{c}	<0.01
24 h HP (MJ/d)	26.0 ± 0.9^{a}	24.2 ± 1.2^{a}	29.1 ± 1.5^{b}	<0.05
Daily energy intake (MJ/d)	29.7 ± 0.7	30.3 ± 0.7	30.5 ± 0.9	0.75
Energy balance (MJ/d)	$3.7 \pm 0.9^{a,b}$	6.1 ± 0.9^{b}	1.4 ± 1.5^{a}	<0.01
RQ	1.09 ± 0.09	1.37 ± 0.13	1.11 ± 0.03	0.13
Metabolic HP (kJ)	496 ± 16	451 ± 19	481 ± 18	0.12
Metabolic energy intake (kJ)	566 ± 11^{a}	564 ± 12^{a}	504 ± 12^{b}	<0.01
$HP/BW^{0.6116}$ (MJ)	1030 ± 34	941 ± 41	1025 ± 41	0.10
Energy intake/$BW^{0.6116}$ (MJ)	1176 ± 23^{a}	1177 ± 24^{a}	1074 ± 26^{b}	<0.05

[1]: n = 5 sows; [2]: n = 4 sows; [a,b,c]: values with different superscripts across a row represent differences $P<0.05$.

Table 5.5 Predicted daily gain of the litter and weight on respiration days of the litter, products of conception, and maternal body

Parameter	Day 30	Day 45	Day 105
Predicted daily gain of litter[1] (g/d)	N/A	8.5	608
Calculated litter weight on respiration day[1] (kg)	N/A	0.37 ± 0.02	12.3 ± 0.8
Calculated weight of products of conception[2] on respiration day[3] (kg)	6.2 ± 0.4	9.3 ± 0.6	21.6 ± 1.4
Calculated maternal BW[3] (kg)	190	193	216
Maternal ME_m HP (MJ/d)	26.5	26.1	28.5
Total BW ME_m HP (MJ/d)	26.5	27.1	30.7
Measured 24 h HP (MJ/d)	26.0	24.2	29.1

[1]: calculated according to McPherson et al, 2004; [2]: defined as the difference between total and maternal gain (i.e. includes litter gain) according to NRC, 1998; [3]: calculated according to NRC, 1998.

5.4 Discussion

Sows (n=5) gained 52.4 ± 5.4 kg between breeding and Day 105 of gestation. The average litter size was 10.4 ± 0.7 piglets. The birth weight of the litters was 16.7 ± 1.1 kg, resulting in an average birth weight of 1.64 ± 0.06 kg/piglet. Daily gain of the sows was not different between breeding and Day 30 or between Day 30 and Day 45, although gain was numerically different between Day 30 and Day 45 (Table 5.4). Daily

gain of sows was greatest in late gestation and, by Day 105, was mostly due to litter gain (Table 5.5)

Digestibility of the individual components of the diet was not different on the three 'days' of gestation, except for the digestibility of fat (Table 5.2). On Day 45, digestibility of fat from the diet was lower than on Day 30. At the same time, the amount of fat in the diet increased between Day 30 and Day 45 of gestation and was maintained through to Day 105. Therefore, the greater fat content of the diet on Day 45 was not digested as completely as on Day 30.

The diet used in this experiment was the same as that used for the entire SRTC breeding herd, therefore feeding of the same batch of diet for the entire study was not possible. Variations in diet composition were not unexpected due to the length of time of the study. The difference in fat digestibility of the diet could be due to the change in diet without sufficient adaptation time or a change in energy metabolism of the sows on Day 45 (McPherson et al, 2004). The apparent digestibility of gross energy numerically decreased on Day 45. HP was also numerically lower on Day 45 than on Days 30 or 105, which suggests a change in energy metabolism occurred in mid-gestation. Close et al (1985) observed that "protein deposition was highest during mid-pregnancy". Kalhan (2000) reported that pregnancy resulted in "an excess of maternal nitrogen retention . . . over that deposited" in the products of conception" and that this change in maternal protein metabolism appeared early in gestation.

Individual amino acids cannot be stored by the body and, therefore, are either incorporated into protein or oxidized. In humans, for example, the labile protein reserve is less than 1% of the total body protein (IOM, 2005). When amino acids are

catabolized by the body, they must be deaminated and the nitrogen excreted from the body to avoid any toxic effects (Wright, 1995). Deamination of excess amino acids and excretion of nitrogenous waste as urea is an energy utilizing process (Shambaugh, 1977; Pattabiraman, 1995; Garrett and Grisham, 1999). Increased nitrogen retention would, on the one hand, increase HP due to the energy utilized to deposit body protein and, on the other hand, decrease HP because ER would increase and energy is not required to excrete nitrogenous wastes. Protein deposition requires an energy input of 44.4 kJ/g of protein deposited and the energy content of body protein is 23.7 kJ/g, therefore 20.7 kJ of energy is released as HP per gram of protein deposited. Garrett and Grisham (1999) reported that the equivalent of four moles of ATP are required to synthesize one mole of urea. A mole of ATP releases 54 kJ in the cellular environment. Therefore, the total cost of urea synthesis is 216 kJ/mol. Urea contains two amino groups; one comes from aspartic acid and the other from ammonium ions as a product, for example, of catabolism of amino acids. The urea cycle serves to remove potentially dangerous ammonia from the body (Garrett and Grisham, 1999). The energy cost to produce urea, per gram of nitrogen excreted, is 7.7 kJ and per gram of nitrogen from excess amino acid catabolism is 15.4 kJ. In summary, the HP of protein deposition is 20.7 kJ/g and the HP of amino acid catabolism is 15.4 kJ/g. In contrast, the HP related to lipid deposition is 13.9 kJ/g of lipid. The RQ throughout gestation was greater than one, thus indicating active storage of body fat through lipogenesis. However, on Day 45, there was a trend toward a RQ greater than on Days 30 or 105 and, coupled with the greater ER, indicates greater lipid deposition.

McPherson et al (2004) dissected fetuses at different stages of gestation and determined that fat and protein gains were limited before day 69 of gestation. After day 69, however, protein requirements for lean-tissue gain increased 19-fold supporting the idea of a two-phase feeding strategy for improved piglet development. In this feeding strategy, the diet would be changed after day 70 of gestation in order to better support the energy and protein requirements of the rapidly growing piglets. The diet before day 70 would be designed to support maternal growth, focusing on body tissue gain to replace that which was lost in the previous lactation. This is imperative to maximize piglet viability and sow longevity.

The daily gain of these sows from breeding to Day 30 of gestation was lower than other production sows because of the experimental treatments applied (see Chapter 3.0) in the period between weaning and rebreeding. Specifically, the sows were fed approximately twice ME_m intake in the seven days before breeding, resulting in rapid growth of almost 1.3 kg/d. In the period immediately after weaning, the sows were fed approximately ME_m, thus resulting in body weight and composition that remained constant from the end of lactation. In fact, however, sow body weight numerically dropped over the study period of seven days. The effects of skip-a-heat breeding (Clowes et al, 1994) and *ad libitum* feeding in the days post-weaning (Brookes et al, 1975) have previously been suggested as methods to improve sow productivity in subsequent parities. Therefore, the experimental procedures were not different compared to industry recommendations. In fact, the total weight gain of the sows was not different than predicted by the current model (NRC, 1998). Sows weighed 186.6 kg at breeding and were predicted to gain 48 kg over the entire gestation. Total measured

weight gain averaged 52.4 ± 5.4 kg. Total gestation weight gain was expected to be lower than predicted due to the greater ME_m (506 kJ/$BW^{0.75}$) compared to the value used in the model (444 kJ/$BW^{0.75}$). However, the average birth weight of the piglets (1.64 ± 0.06 kg) exceeds recent reports of birth weights. Grandinson et al (2002) reported that for piglets born between 1984 and 1999, the average birth weight was 1.45 ± 0.3 kg. Varona et al (2007) reported average birth weights of 1.36 kg for Landrance and 1.30 kg for Yorkshire piglets. Therefore, total gestation weight gain would be expected to be greater by at least 3 kg due to higher average piglet birth weight alone.

A comparison of energy intake versus HP, expressed per unit metabolic body weight, showed that energy intake decreased on Day 105 of gestation due to body weight increase. Energy intakes on Days 30 and 45 were greater than HP, but HP was similar to energy intake on Day 105. Therefore, sows in late gestation were close to negative energy balance, which agrees with the observations of Close et al (1985). The energy in excess of the maintenance energy requirement (ME_m) can be used for growth of the sow body and conceptus products. According to the data of McPherson et al (2004), 0.7 to 3.5 MJ of energy is deposited to fetal piglet growth daily. Therefore, the excess of less than 2 MJ/d would be barely adequate to support piglet growth in late gestation. Previous observations (Cole, 1990; Walker and Young, 1992) have shown that sows in late gestation will become catabolic and mobilize body lipid for energy.Therefore, it is advisable to increase energy intake to avoid catabolism in late gestation.

However, increasing the energy intake of sows in late gestation to avoid catabolism of body tissue often results in depressed feed intake during lactation, thus

leading to a more significant catabolic state (Miller et al, 2000) and reduced sow longevity (Rodriguez-Zas et al, 2006). Increasing feed intake during late gestation to improve piglet birth weight is only effective when applied over at least two consecutive parities. This suggests a long-term improvement of body condition of the sow, rather than an immediate effect of the increased feed intake on the piglets, is an effective feeding strategy (Miller et al, 2000).

Piglet requirements are small compared to the needs of the sow (Wenk et al, 1980; Walker and Young, 1992). A comparison of the body weight of piglets versus the sow is possible by using the prediction equations of McPherson et al (2004) (Table 5.4). On day 45, calculated piglet weight was 370 g compared to the sows' body weight of 202.4 kg or less than 2%. On day 105, calculated piglet weight was 12.3 kg compared to the sows' body weight of 237.5 kg and, therefore, 5.2 % of the measured body weight. It is possible to predict the daily HP of sows for zero ER by using the ME_m value previously calculated (Chapter 3.0). The calculated ME_m value of 506 $kJ/BW^{0.75}$ was determined using the formulae of Lodge et al (1979) and includes an assumed efficiency of energy utilization (k_{pf}) of 0.70 (Verstegen, 1971) for pregnant sows. Thus ME_m represents the energy required for the maintenance of body weight and composition with zero net energy retention (i.e. energy intake is exactly equal to HP). A comparison of the predicted HP to the measured HP on a total body weight basis shows a large difference in mid-gestation and a smaller difference in late gestation where the predicted HP is greater than what was actually observed. Repeating the comparison using only the maternal BW (calculated by subtracting from the total BW the weight of the products of conception) yields predicted HP values above, on Day 45, and below, on

Day 105, the measured HP. The products of conception (9.3 kg) are 4.8 % of the total BW of the sow on Day 45. Therefore, it was expected that their contribution to the measured HP would not detected. There are two possible explanations for the lower HP on Day 45. One, which was discussed previously, is that protein metabolism changed thus reducing the energy required for nitrogenous waste disposal and increased ER. Secondly, a change in hydration of the uterine or maternal tissues may have occurred thus increasing BW, but not energy metabolism (Good, 1979). By Day 105, the products of conception (21.6 kg) are 14.2 % of the total BW of the sow. The predicted HP of the sow only is lower than the measured HP and this suggests that the products of conception have an influence on the total measured HP and, therefore, ER. Data by McPherson et al (2004) suggested that fetal piglets require 0.7 to 3.5 MJ/d to support growth. Some of the energy above ME_m would be retained in the process of tissue deposition and some would be utilized to deposit that body tissue.

Often the energy metabolism of sows is expressed on the metabolic live weight basis with the standard exponent of 0.75. However, a more appropriate exponent may exist to correlate HP and BW. In this experiment, the exponent was calculated (see 5.2.3) to be 0.6116. This value is not different than the value of 0.60 proposed by Noblet et al (1985).

Body weight gain, HP, and RQ of pregnant sows were different on Days 30, 45, and 105 of gestation. Therefore, the null hypothesis "body weight gain, diet digestibility, heat production, and respiratory quotient of gravid sows will not be different at early-, mid-, and late-gestation and will not vary with products of

conception" can, in majority, be rejected. Diet digestibility was not different, so therefore that component of the null hypothesis cannot be rejected.

5.5 Conclusions and implications

Energy intake in late gestation was only slightly greater than HP and the difference was insufficient to support calculated fetal growth. Therefore sows in late gestation require greater energy intakes to ensure sufficient energy for optimal maternal and fetal development and avoid catabolism of body tissue (Cole, 1990). Under current energy and protein intake recommendations (NRC, 1998; Aherne et al, 1999), sows need to have sufficient body tissue energy reserves to support lactation because they cannot consume enough dietary energy. Deposition of body tissue must occur before late gestation to avoid impacts on lactation feed intake (Miller et al, 2000). This is especially important for modern sows due to increased number of litters per year, piglets born per litter, and daily weight gains of those litters (Ball et al, 2008). Greater energy demands for production are coupled with the body composition changes in sows leading to increased lean tissue mass per live weight (Wenk et al, 1980; Merks, 2000; Brown-Brandl et al, 2004). As a result, the ME_m has increased due to the greater lean tissue content and greater energy demands of whole-body protein turnover for both the sow and her litter.

5.6 Literature cited

Aherne, F. X., G. R. Foxcroft, & J. E. Pettigrew. 1999. Nutrition of the Sow. Pages 1029-1044 in Diseases in Swine. Straw, B. E., S. D'Allaire, W. L. Mengeling & D. J. Taylor, eds. 8th ed. Iowa University Press, Ames, Iowa.

Brookes, P. H., D. J. A. Coles, P. Rowlinson, V. J. Croxson, & J. R. Lucscombe. 1975. Studies in sow reproduction. 3. the effect of nutrition between weaning and remating on the reproductive performance of multiparous sows. Anim. Prod. 20: 407-412.

Engblom, L., N. Lundeheim, A. Dalin, & K. Andersson. 2007. Sow removal in swedish commercial herds. Livest Sci. 106: 76-86.

Good, W. 1979. Water relations of fetal development. Med. Hypotheses. 5: 859-875.

Institute of Medicine. 2005. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington, D.C., National Academy Press.

Jensen, H., & B. Peet. 2006. 30 pigs per sow per year - are we there yet? Advances in Pork Production. 17: 237-243.

Kalhan, S. C. 2000. Protein metabolism in pregnancy. Am. J. Clin. Nutr. 71(suppl): 1249S-1255S.

Lucia, T., G. D. Dial, & W. E. Marsh. 1999. Estimation of lifetime productivity of female swine. J. Amer. Veterinary Medical Assoc. 214: 1056-1059.

McPherson, R. L., F. Ji, G. Wu, J. R. Blanton, & S. W. Kim. 2004. Growth and compositional changes of fetal tissues in pigs. J. Anim. Sci. 82: 2534-2540.

Miller, H. M., G. R. Foxcroft, & F. X. Aherne. 2000. Increasing food intake in late gestation improved sow condition throughout lactation but did not affect piglet viability or growth rate. Anim. Sci. 71: 141-148.

Noblet, J., J. Y. Dourmad, & M. Etienne. 1990. Energy utilization in pregnant and lactating sows: Modeling of energy requirements. J. Anim. Sci. 68: 562-572.

Rodriguez-Zas, S. L., C. B. Davis, P. N. Ellinger, G. D. Schnitkey, N. M. Romaine, J. F. Connor et al. 2006. Impact of biological and economic variables on optimal parity for replacement in swine breed-to-wean herds. J. Anim. Sci. 84: 2555-2565.

Walach-Janiak, M., S. Raj, & H. Fandrejewski. 1986. Protein and energy balance in pregnant gilts. Livest. Prod. Sci. 15: 249-260.

Walker, B., & B. A. Young. 1992. Modelling the development of uterine components and sow body composition in response to nutrient intake during pregnancy. Livest. Prod. Sci. 30: 251-264.

6.0 GENERAL DISCUSSION AND CONCLUSIONS

6.1 Introduction

The primary objective of this thesis was to examine the effects of certain physiological states on energy metabolism of sows. Sows are the foundation of pork production, yet little research has focused on sow energy metabolism. The modern, high producing sow spends her entire productive life either pregnant or lactating. These physiological states create unique demands on the metabolism of the sows. NRC (1998) proposed a factorial method to determine energy and protein requirements of pregnant and lactating sows in addition to maintenance requirements. This thesis aims to assess the impact on energy metabolism of: 1) feeding level and frequency on non-pregnant sows and 2) advancing pregnancy.

Indirect calorimetry and nutrient digestibility procedures were used to measure energy metabolism. Comparisons of energy metabolism were made between feeding levels [Chapter 3.0], feeding frequency [Chapter 4.0], and advancing pregnancy [Chapter 5.0].

6.2 Changes of energy metabolism

In the first study [Chapter 3.0] the effect of feeding level was investigated in non-pregnant sows. Regardless of the energy intake, 473 or 925 kJ ME/$BW^{0.75}$, representing approximately 1 times and 2 times ME_m intake, respectively, the calculated ME_m for these sows was 506 kJ ME/$BW^{0.75}$. This value is 10% higher than previously reported (ARC, 1981; NRC, 1998). The greater ME_m value has been attributed to

changes in body composition of modern, high producing sows (Brown-Brandl et al, 2004) and the relative effect of protein turnover on total HP (Wenk et al, 1980). Daily HP was greater in sows fed 925 kJ ME/$BW^{0.75}$, but not twice as great, because energy was retained as protein and fat. Therefore, energy intake had an effect on total daily HP, but no effect on measured ME_m.

We hypothesized that the experimental feeding regimen proposed by Moehn et al. (2004) would not affect the total daily HP of sows. In the second study [Chapter 4.0] the effect of this experimental feeding regimen was investigated in non-pregnant sows. The HIF was measured. Although there were measured differences in HP between frequent feeding and meal feeding, the effect on total daily HP was not appreciable. Therefore, the experimental feeding regimen proposed by Moehn et al. (2004) can be used in future studies of energy and protein metabolism because 24 h HP can be predicted from short term measurements with frequent feeding.

Finally, in the third study [Chapter 5.0], the effect of pregnancy on energy metabolism was investigated. The HP and RQ of sows were measured on days 30, 45, and 105 of gestation. Pregnancy did not affect HP more than could be explained by the increase in BW, as shown previously (Lodge et al, 1979). Sows were fed the same dietary intake throughout gestation as recommended by Aherne et al (1999). As a result, sows in late gestation were nearly underfed to maintain positive energy balance. However, as shown by a RQ greater than one on day 45 and backfat measurements, body fat was previously stored in gestation. It seems more appropriate to feed sows correctly for their metabolic demands once the demands are imposed. The alternative, and currently used method, forces the sow body to store excess energy through mid-

gestation to be used to supplement the energy demands of late gestation and, perhaps, lactation.

6.3 Application of ME_m value

In the first study [Chapter 3.0], the sows were fed 925 kJ ME/$BW^{0.75}$ intake. This translated into 3.69 ± 0.05 kg of feed per day with a lysine content of 0.65%. The product of these values is 23.97 ± 0.35 g of lysine per day intake. The maintenance requirement for lysine is estimated at 36 mg/$BW^{0.75}$ (NRC, 1998), but more recently has been determined to be 49 mg/$BW^{0.75}$ (Samuel et al, 2008). Therefore the amount of lysine available for protein accretion above the maintenance requirement was 21.5 ± 0.3 g/d. Given that body protein is 7.08 % lysine (Möhn et al, 2000), this lysine intake would represent accretion of 304 ± 5 g/d of protein. Based on an energy cost of protein accretion of 44.4 kJ ME/g (NRC, 1998), 13.5 MJ ME would be utilized for a protein accretion of 304 g/d. Total daily ME intake was 46.07 MJ; less the energy for ME_m (180 $kg^{0.75}$ * 0.506 MJ/$BW^{0.75}$) of 25.5 MJ ME leaves 20.6 MJ available for growth. Subtracting the energy cost of protein accretion of 13.5 MJ from the energy available for growth (20.6) leaves 7.1 MJ ME per day available for fat accretion. Because the energy cost of fat accretion is 52.3 kJ/g of fat, this excess energy (7.1 MJ) would result in 135 ± 3 g/d of fat accretion. In summary, the composition of gain calculated in this manner would be 304 g/d protein and 135 g/d fat for a total of 438 g/d. Protein is 75% water, therefore 304 g/d of protein would total to 1215 ± 18 g/d of lean tissue. Likewise, fat is 10% water, therefore 135 g/d of adipose tissue would total to 150 ± 4 g/d. The total body weight gain would be 1215 g/d of protein plus 150 g/d adipose tissue for total

gain of 1364 ± 21 g/d, which is numerically greater than the measured gain of 1292 ± 215 g/d. Therefore, use of the ME_m value from this thesis [Chapter 3.0] and the recently determined lysine maintenance requirement value (Samuel et al, 2008) reduced the over-estimate of the predicted versus the measured weight gain of these sows. However, the effect of physical activity outside of the respiration chamber on energy metabolism could not be estimated and, therefore, could explain any differences.

6.4 Conclusion

Sow energy metabolism was investigated for non-pregnant sows fed approximately ME_m and twice ME_m. A new ME_m value of 506 kJ ME/$BW^{0.75}$ was calculated and is proposed to replace current estimates (ARC, 1981; NRC, 1998). Non-pregnant sows were also fed a unique experimental feeding regimen proposed by Moehn et al (2004) as ideal for studies of energy and protein metabolism. The feeding regimen did not induce measurable changes in the daily energy metabolism of sows. Therefore this regimen is justified for use in future studies. Measurements of energy metabolism of sows during gestation revealed that energy was stored during mid-gestation and used to support late gestation energy requirements. Therefore, a more appropriate feeding program should be developed.

6.5 Literature Cited

Agricultural Research Council. 1981. The nutrient requirements of pigs. 1st ed. Slough, U.K, Commonwealth.

Aherne, F. X., G. R. Foxcroft, & J. E. Pettigrew. 1999. Nutrition of the Sow. Pages 1029-1044 in Diseases in Swine. Straw, B. E., S. D'Allaire, W. L. Mengeling & D. J. Taylor, eds. 8th ed. Iowa University Press, Ames, Iowa.

Brown-Brandl, T. M., J. A. Nienaber, H. Xin, & R. S. Gates. 2004. A literature review of swine heat production. T. ASAE. 47(1): 259-270.

Lodge, G. A., D. W. Friend, & M. S. Wolynetz. 1979. Effect of pregnancy on body composition and energy balance of the gilt. Can. J. Anim. Sci. 59: 51-61.

Moehn, S., R. F. P. Bertolo, P. B. Pencharz, & R. O. Ball. 2004. Pattern of carbon dioxide production and retention is similar in adult pigs when fen hourly, but not when fed a single meal. BMC Physiology. 4: 11-18.

National Research Council. 1998. Nutrient requirements of swine. 10th ed. Washington, D.C., National Academy Press.

Samuel, R. S., S. Moehn, P. B. Pencharz, & R. O. Ball. 2008. Dietary lysine requirement for maintenance is 49 mg/kg$^{0.75}$ in a population of modern, high producing sows. Advances in Pork Production. 19:A1.

Wenk, C., H. P. Pfirter, & H. Bickel. 1980. Energetic aspects of feed conversion in growing pigs. Livest. Prod. Sci. 7: 483-495.

APPENDIX

A.1 Introduction

Respiration data was collected using sensors purchased from Qubit systems (Kingston, Ontario, Canada) arranged as shown in Figure A.1. Air was withdrawn from the respiration chambers by vacuum pumps and a sub-sample was forced through the analyzers. It was, therefore, necessary to ensure that the sub-sample was representative of the total air withdrawn from the respiration chambers. Two tests were performed, as discussed below: 1) nitrogen injection to test the oxygen sensors, and 2) CO_2 release from $NaHCO_3$ solution by concentrated $HCl_{(aq)}$, to validate the calorimetry system.

A.2 Calorimetry system validation

The efficiency of the system was tested before any studies began. Firstly, the oxygen sensors were tested by injecting a measured flow of nitrogen over a known time into the sealed respiration chambers. The total injected nitrogen was calculated and compared to the reduction in measured oxygen by the sensors. The efficiency of this test was 102.6 ± 0.6% for the oxygen sensors over repeated (n=6) measurements. The linearity of the response of the O_2 analyzers was tested using gases of various known O_2 concentrations. The analyzers responded linearly ($R^2 > 0.998$, CV < 0.35%) to a series of gases with O2 contents between 0 and 21%.

Secondly, the carbon dioxide sensors were tested by dissolving a known quantity of $NaHCO_3$ into water. A solution was placed into each of the respiration chambers and fitted with an extension set that would deliver concentrated $HCl_{(aq)}$ from a syringe driven by an injection pump. The chambers were sealed and the syringe pumps

were started. As the $HCl_{(aq)}$ was delivered, CO_2 was evolved. The amount of CO_2 measured by the sensors was quantified and compared to the quantity of $NaHCO_3$ added. The recovery of CO_2 was 107.3 ± 2.1% over repeated (n=15) measures for both chambers. The CO_2 analyzers response to changing CO_2 content of the air was a non-linear response curve. The curve was repeatedly (n=5) determined using gases of known CO_2 concentration (0.04, 0.08, 1.2, and 1.5%). The corrected CO_2 concentrations were calculated in Excel using the most current non-linear response curve:

$$CO_2 = -0.18653x^5 + 1.563x^4 - 4.9975x^3 + 7.8458x^2 - 5.5865x + 1.4242$$

A.3 Oxygen sensor details and the effect of temperature

The oxygen sensors contain a fuel cell which counts the molecules of oxygen in the volume of air passing through the sensor. The oxygen sensor fuel cell has pressure compensation, but not temperature compensation. The oxygen sensors are heated, but the heating is insufficient to correct for changes in air temperature. Therefore, the room air was maintained between 19 – 23 °C by the ventilation system. This range of temperature would impact oxygen measurements by at most 1 percentage unit. However, as discussed previously, during the overnight period for the experiments of Chapters 3.0 and 4.0, the temperature control failed to maintain the desired range. During this period, errors in oxygen measurements were greater than expected and, therefore, are not representative of the true energy metabolism. Since these experiments, the room air temperature control has been repaired and temperature correction has been added in-line with the sensors and simultaneously recorded by the data acquisition software.

Figure A.1 Schematic of the indirect calorimetry system